The Journal of
CLINICAL
ENDOCRINOLOGY

VOLUME 11 FEBRUARY, 1951 NUMBER 2
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CORTISONE *PLUS* INSULIN IN THE PALLIATIVE TREATMENT OF RHEUMATOID ARTHRITIS: A PRELIMINARY STUDY

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IN THE months that have elapsed since the first description by Hench, Kendall, Slocumb and Polley (1) of the clinical effect of cortisone in rheumatoid arthritis, several confirmatory studies (2, 3, 4, 5, 6) have appeared, and most authors are in agreement that large doses are mandatory in the primary treatment of the disease. Quantities as great as 300 mg. of cortisone have been given initially (1, 3), and the usual schedule of active treatment requires 100 mg. daily. Because of the high cost of this compound, and because of its potential and actual adverse effects upon physiology, we have inquired into the possibilities of a method of treatment in which smaller quantities would suffice for a comparable degree of clinical palliation.

Laboratory experiments (7, 8) indicate that adrenocortical tissue *in vitro* is capable of converting a number of 21-carbon steroids lacking an oxygen at carbon 11 to steroids having this configuration. One such study (7) indicates that insulin facilitates this process From these results we were

Received for publication August 19, 1950.

led to a further consideration of the physiologic and pharmacologic role of cortisone and the question of the intimate character of its effects upon general tissue cells, and the possible intercession of hormones such as insulin. Our reflections prompted a preliminary clinical trial of cortisone plus insulin which has yielded interesting and useful data, not only suggesting improvements in therapy but also possibly bearing out some concepts of the action of cortisone.

CLINICAL

Cortisone and insulin were administered to 12 hospitalized patients with typical rheumatoid arthritis. The patients included 9 women and 3 men, aged 28 to 67 years, in stages I to IV of the disease (9). The duration of illness ranged from six weeks, in a young woman of 28, to twenty years in a 49-year-old man who was our most refractory subject. The distribution of the joint lesions was typical, and varied from fusiform swelling of two metacarpophalangeal joints and two proximal interphalangeal joints, in 1 case, to widespread disease of the major and minor joints, including spondylitis, in 2 cases. (When spondylitis was present, however, it was part of otherwise typical, generalized rheumatoid arthritis, no patient being included who had spondylitis alone.) A variety of procedures had previously been employed to treat these patients over the years, except the young woman aged 28, referred to above, who had had no previous treatment. In every case the last prior treatment had been remitted for a sufficient time to permit of well-established relapse, so that the effects of the present procedure could be distinguished without interference.

METHODS

All patients were hospitalized for the entire period of the study. All were part of a larger group in which numerous steroid compounds were being studied, and at no time during this work did the patients, the nurse in attendance, the internes or the residents know what medication was being administered, in what concentration, or at what dosage, as plain bottles with coded labels were employed both for the cortisone and insulin. Following an initial pretreatment period of observation of from two to seven days for the determination of base-lines and for appraisal of the disease process in an untreated condition, the patients were divided without selection into 3 groups of 4 patients each, designated and treated as follows:

Group A. For ten days these patients received a single daily injection of 12.5 mg. of cortisone and 20 U. of plain insulin, at 9 A.M., the two materials being mixed in the same syringe and injected intramuscularly. This was followed by single daily injections of 50 mg. and 20 U., given in the same manner, for ten days; and then by 50 mg. and 40 U. daily, divided into two doses at 9 A.M. and 4 P.M., for ten days.

Group B. These patients received 25 mg. of cortisone and 40 U. of plain insulin daily, divided into two doses given at 9 A.M. and 4 P.M., for ten days, and then 50 mg. and 40 U. daily given in the same manner for two ten-day periods. The two materials were mixed in the same syringe and injected intramuscularly.

Group C. These patients received 25 mg. of cortisone and 40 U. of plain insulin daily, divided into two doses given at 9 A.M. and 4 P.M., for ten days; then 50 mg. and 40 U. daily were given in the same manner for ten days; and finally 50 mg. and 60 U. for ten days. In this group the cortisone was given intramuscularly in one injection and the insulin was injected subcutaneously in another site.

The object of treating groups B and C differently during the first twenty days was to distinguish any effect, or the lack of it, that could be ascribed to the co-presence of the two agents in the same menstruum and in the same site of injection.

For clinical reasons these schedules were modified upon occasion: the full dosage of insulin was not begun in some cases until cortisone had been given for one day; and in others, the amount of insulin was reapportioned between morning and evening doses to avoid hypoglycemic reactions. Charts A, B and C exhibit the actual doses administered to patients in the three groups.

Routine laboratory procedures were employed. Sedimentation rates were determined by the Westergren method, with correction for the degree of anemia present, and are expressed in millimeters in one hour. The normal sedimentation rate according to this method is 10 mm. or less (10).

The materials used were cortisone acetate, Δ4-pregnene-17α, 21-diol-3, 11, 20-trione 21-acetate, in aqueous suspension of 25 mg. per cc., and "plain" insulin (Lilly) dissolved in water, 40 U. per cc.

The control of hypoglycemic symptoms, which occurred one or more times in every patient, required the oral administration of sweetened orange juice. This was always done promptly; no effort was made to prolong the hypoglycemia, nor did we desire to produce anything resembling insulin-shock for the purpose of "stimulating the adrenals" (11).

RESULTS

The pertinent findings are summarized in Table 1, in which Roman numerals are used to designate features of the disease in accordance with the uniform criteria of the New York Rheumatism Association (9).

For convenience, these criteria are recapitulated, in condensed form, as follows: (For the strict manner of their application, the full statement (9) should be consulted.)

Stage of the disease. I—osteoporosis may be present but no destructive changes; II—osteoporosis, slight destruction of bone or cartilage with some atrophy of adjacent muscles; III—osteoporosis, destruction, extensive muscular atrophy, and deformity of joints; IV—the same as III with fibrous or bony ankylosis.

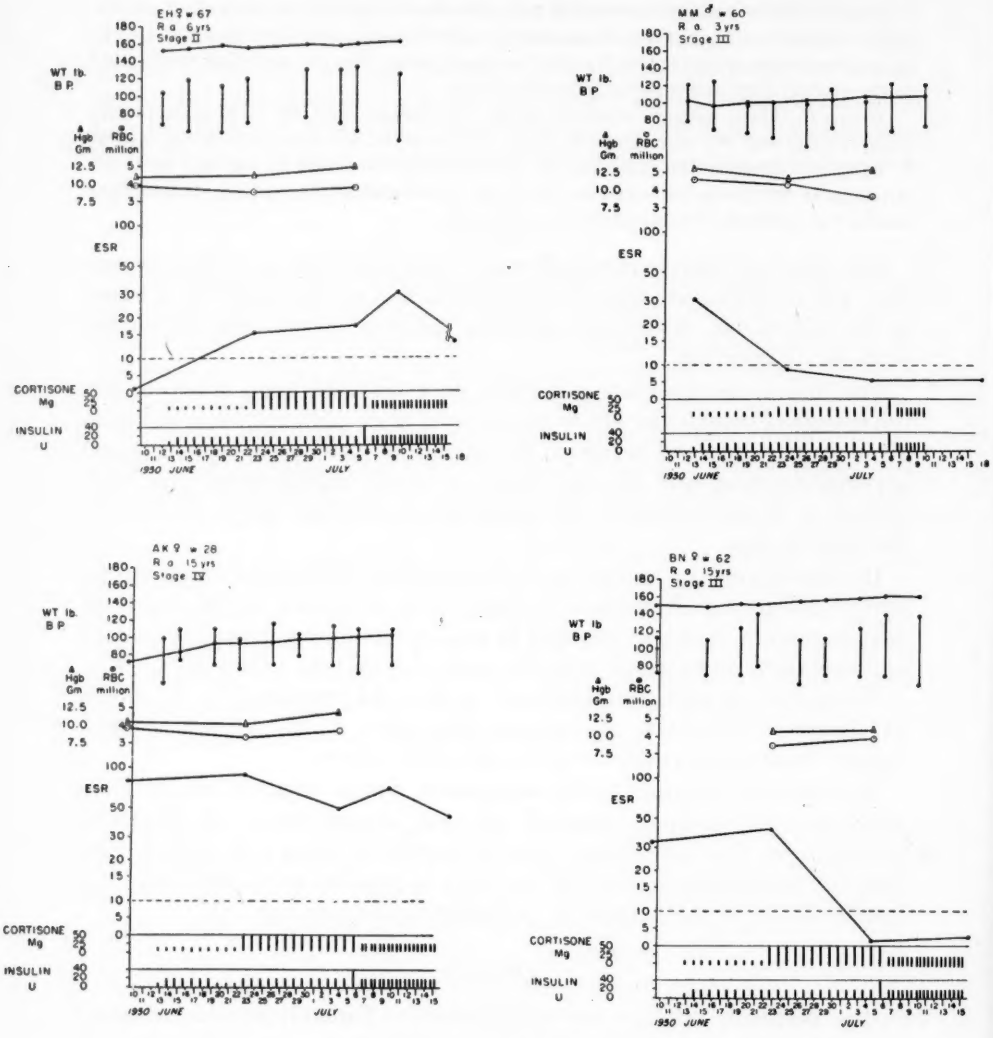

CHART A

Functional capacity. I—unimpaired; II—normal activities despite discomfort or limited motion; III—limited, foregoing usual occupation but caring for self; IV—bedridden, largely incapacitated even for self-care.

Grade of response to therapy. I—complete remission including normal sedimentation rate; II—major improvement with minimum residual joint signs, and deformity due to irreversible changes; III—minor improvement, but no articular or extra-articular activ-

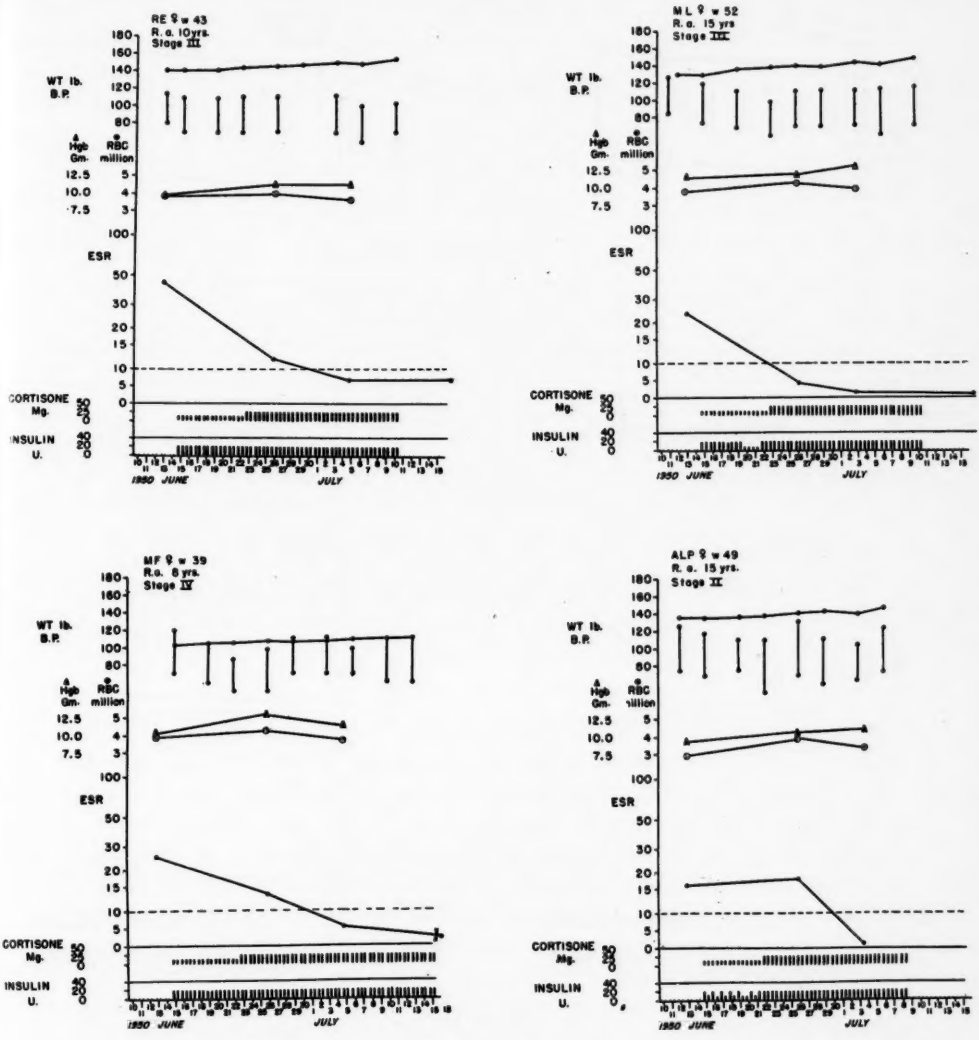

CHART B

ity in new sites, and decrease in signs of activity; IV—no progress, with roentgenologic evidence of advancing disease.

We apply the term "results" to the findings immediately following the treatment period. Experience has made it clear that continuing study of the same patients, after cessation of effective cortisone therapy, will disclose

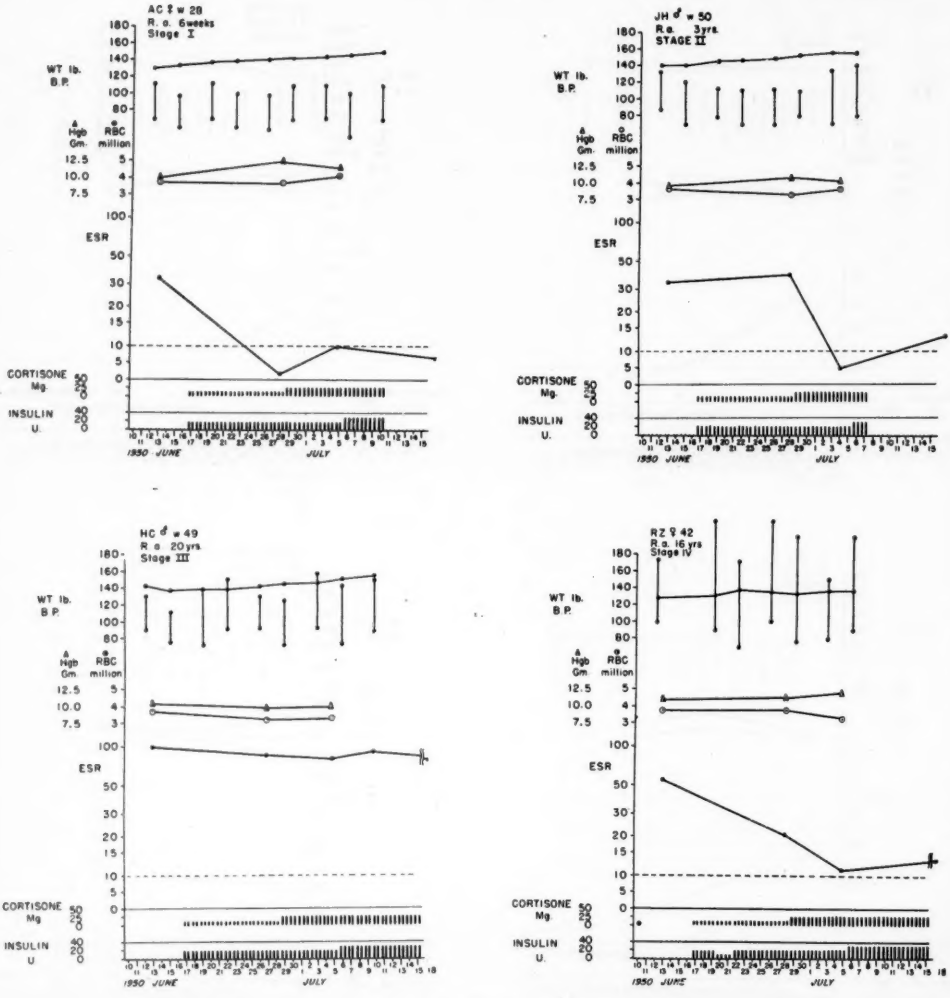

CHART C

eventual relapse at some earlier or later time, and we have no reason to suppose that the present procedure will have conferred more lasting effects than any other of the recent cortisone procedures.

The over-all results refer to the dosage of 50 mg. of cortisone and 40 U. or more of plain insulin, daily, to which all patients were ultimately advanced. They consist of complete remission (Grade I response) in 7 cases; major improvement (Grade II response) in 4; and minor improvement

TABLE 1

Patient, Age, Sex	Stage of disease	*Treatment*, cortisone +insulin ×days	Date	Body wt. (lbs.)	B.P., mm. Hg.	RBC (10^6)	Hgb. (Gm.)	WBC (10^3)	PMN, %	ESR, mm./hr.	Glucose tolerance test (hours) F	½	1	2	3	4	5	Funct. capac., class	Result, grade
			(1950)																
EH 67 f	III	12½ mg.+ 20 U.×10	6/12 Before	156	110/75	3.9	11.0	15.0	70	1	107	191	222	211	124	111	66	III	
			7/12 After	164	126/50	3.8	12.3	10.0	70	30	103	102	187	167	185	154	91	II	II
AK 28 f	IV	50 mg.+ 20 U.×10	6/9	75	116/72	3.9	11.5	11.5	67	80	89	141	168	137	105	125	93	IV	
			7/11	104	110/70	3.7	11.8	15.7	61	45	84	123	152	114	120	121	75	III	II
MM 60 m	III	50mg.+ 40 U.×10	6/9	102	122/80	4.5	12.5	15.4	59	30	93	172	203	221	158	65	82	III	
			7/10	108	118/70	3.8	12.7	6.0	73	5								II	I
BN 62 f	IV		6/8	150	112/74	3.5	10.8	6.1	58	32	94	164	170	168	132	111	76	IV	
			7/12	160	138/60	3.9	11.1	5.8	76	3	100	100	148	204	157	73	56	III	II
RE 43 f	III	25 mg.+ 40 U.×10	6/14 Before	140	114/80	3.9	9.7	7.2	66	45	94	148	156	151	127	179	88	II	
			7/10 After	154	104/70	3.7	11.3	9.9	60	7								I	I
MF 39 f	IV	50 mg.+ 40U.×20	6/13	103	120/70	3.9	10.4	9.3	69	25	94	131	136	102	84	79		III	
			7/15	110	110/60	3.8	11.5	10.3	83	5								II	I
ML 52 f	III		6/14	120	128/86	3.9	11.3	6.5	52	23	94	154	138	143	107	77	85	II	
			7/10	148	114/70	3.9	12.6	8.7	65	1								I	I
ALP 49 f	II		6/13	135	126/76	3.0	8.8	5.1	67	16	91	166	177	105	77	67		II	
			7/8	143	124/74	3.4	11.0	17.0	75	2								I	I
AC 28 f	I	25 mg.+ 40 U.×10	6/15 Before	130	112/70	3.8	10.0	9.0	66	35	77	147	134	79	125	97	113	II	
			7/10 After	149	110/70	4.1	11.5	12.1	72	7								I	I
HC 49 m	IV	50 mg.+ 40 U.×10	6/16	143	136/90	3.7	10.3	9.0	69	100	86	148	200	168	173	98		IV	
			7/13	147	150/85	3.2	9.7	9.8	66	88	89	100	140	215	160	105	77	III	III
JH 50 m	II	50 mg.+ 60 U.×10	6/16	140	132/88	3.7	9.5	12.4	64	35	92	161	124	98	103	91		II	
			7/4	155	138/80	3.7	10.3	14.7	70	5								I	I
RZ 42 f	IV		6/15	128	174/100	3.8	11.0	9.6	73	55	93	170	166	134	113	96	102	IV	
			7/5	136	180/90	3.3	12.0	9.4	77	6								III	II

(Grade III response) in 1. *Under the same criteria, in the same circumstances and in the same hands, comparable patients have attained to about the same degree of benefit while receiving daily 100 mg. or more of cortisone alone.*

Cortisone 12.5 mg., insulin 20 U. Except for a decline in the sedimentation rate in 1 patient (MM), possibly fortuitous, none of the patients treated at this level showed objective clinical benefit or improvement in terms of laboratory findings during the ten-day period of study; in fact, in the remaining 3 patients the sedimentation rate increased.

Cortisone 25 mg., insulin 40 U. In 6 of the 8 patients starting treatment at this level, there was an abrupt fall in the sedimentation rate, but it was not clear at the end of the first ten-day period that conspicuous clinical improvement was taking place. It was only after changes in the joints became more obvious that it was realized improvement could be traced back to this level of dosage, in good correspondence with the decline in sedimentation rate. Of the 6 patients, 4 had undoubted clinical improvement during the 25 mg. period.

Cortisone 50 mg. Whether 20 or 40 U. of insulin were given daily, at this level the sedimentation rates of 9 of 12 patients steadily fell to normal

within the first ten-day period or soon thereafter. One patient (EH) improved by clinical criteria during early treatment despite a rising sedimentation rate which, with continued treatment, returned to normal. We have not infrequently observed a coincidental ESR rise when a patient started walking with severely affected weight-bearing joints. One other patient (HC) had an ESR which was initially 100, but which dropped to 88 after thirty days' treatment, and later to 56. The same factor, increased walking, may have played a part in the slow reduction of the ESR in this case. *It would appear significant that this man obtained as complete improvement as he had previously shown on full doses of cortisone for one month earlier in the year and that, although his sedimentation rate was reduced to 32 while on full doses of cortisone alone and at complete bed rest, it quickly rose to 72 when he started walking.*

After twenty days Group C was chosen for a further trial of 50 mg. of cortisone with 60 U. of insulin daily for ten days. It did not seem that the higher insulin dosage was any more effective than the lower dosage.

The results failed to reveal any difference that might be due to the administration of cortisone and insulin in a single intramuscular injection versus the injection of the two substances separately.

Other features. As expected, all of the patients gained weight, one as much as 29 pounds in a 30-day period. There were normal variations in the blood pressure, inconstantly in either direction. Red cell counts and hemoglobin varied somewhat but, in general, showed neither a decline implying hemodilution nor a marked increase such as is sometimes reported during the use of cortisone (3). White cell counts and differential counts failed to show any trend.

In our earlier report (12) we called attention to 1 patient (RE) in whom a slight edema of the ankles developed while on cortisone-insulin therapy. A careful study of her history disclosed that she had had several such episodes during the previous few years, in no way related to any therapeutic procedures. We assume, therefore, that a recurrence of this edema should not be attributed to cortisone-insulin.

Pretreatment fasting blood sugars were within normal limits in all patients. In oral glucose tolerance tests, using 100 Gm. of glucose in a single dose, we were impressed by the fact that 10 of the 12 patients showed a considerable delay in the return of blood sugar to the fasting level: in 7 patients a period of four to five hours was required. None of the patients was known to be diabetic or had glycosuria even in connection with the glucose tolerance test. Our experience and that of others (3) show that rheumatoid arthritis is rarely associated with diabetes.

There is no general agreement as to the finding of abnormal glucose

tolerance curves in patients with rheumatoid arthritis. Coggeshall (13) states that "the glucose tolerance is normal in over 85 per cent of the patients (citing Kersley (14)). When the disease is severe there may be only a slight rise and delayed fall up to four hours. It may be similar to the prediabetic curve." Reference to the original study by Kersley (14) shows that although this author noted some abnormal curves, the average of a large number of cases was normal. On the other hand, Pemberton (15) found "about sixty per cent of arthritics" to show what he described as a "delayed sugar removal." He felt that although this phenomenon was encountered in arthritis, it might apply to the underlying inflammatory process or be an expression of inflammation in general (like leucocytosis). Copeman (16) refers to Pemberton's findings but adds that "this has not been the experience of all who have attempted to repeat this work. The general consensus is that where diminished sugar tolerance occurs it is a secondary effect." Freyberg (17) states that, in his experience, of all patients with rheumatoid arthritis so studied, about 50 per cent show an abnormal glucose tolerance curve.

In the present series an impairment was noted in a majority of patients, but in view of the small number involved and the vagaries of the oral glucose tolerance test, we defer any interpretation of these findings. During treatment with cortisone and insulin, the fasting blood sugar was unchanged and the tolerance curves became either slightly higher or lower than they were initially, entirely without relation to the clinical progress of individual patients.

Hypoglycemia usually produced symptoms on the first or second day of treatment with insulin or on increasing the dose of insulin, generally following the morning injection. The value of blood glucose was always found to be within normal limits one and one-half to two hours after insulin injection, so that the hypoglycemia was evidently abrupt in development, as in patients who receive plain insulin without cortisone. Neither the carbohydrate action of cortisone nor its expected influence upon tissue resistivity (18) served to prevent moderate hypoglycemic symptoms, but after about two days the patients achieved a sort of tolerance and were not further disturbed. This may have been due to a larger intake of food, or it may have resulted from the action of a depot of cortisone. It occurred equally in patients receiving separate and combined injections. Some patients who reacted to 10 U. of insulin during the first few days of treatment later tolerated 60 U. without reactions of any kind. Patients with more frequent hypoglycemic episodes were not differently distributed among those responding favorably or unfavorably, nor were patients with less frequent episodes.

The detailed relation among dosage, sedimentation rate and clinical evaluation is shown for each patient in Charts A, B and C.

Later observations show that the use of *protamine zinc insulin*, when substituted for plain insulin in separate injections, was uniformly unsuccessful, and during a week's time almost every patient became worse. The use of *plain insulin alone* in doses of 40 U. daily likewise was followed by a marked recrudescence of the arthritis in 3 out of 4 patients. In order to appraise the effect of *cortisone alone* in the low-range dosage we have employed, 3 of the patients in the present series were given 50 mg. daily for one month or longer. The results confirm those reported by Boland and Headley (3), namely, that there is moderate benefit, at least initially, but after ten to fourteen days in 2 of our 3 cases this was followed by definite regression. In other cases not reported in detail here, receiving varying smaller daily doses of cortisone alone, it eventually became necessary to increase the dosage to 100 mg. daily, or to introduce a daily dose of insulin. Even then, the course varied. These comparisons affirm our belief that a considerable advantage is gained by the combined use of cortisone and insulin, and also our belief that the entire program, with or without insulin is, at best, palliative. In the majority of cases the treatment is interminable, the patient being in no better permanent status after months of therapy than at the outset. It appears from our limited experience that intermittent use of this combination of hormones may conceivably abate the activity in very early cases, so that progression leading to irreversible damage to the joints may be postponed. As yet we cannot show that this actually occurs.

DISCUSSION

Several explanations have been attempted for the action of cortisone (*see* review[1]). In the main they have been extensions of some older thesis, such as antigen-antibody reactions, for which in turn there has not been satisfactory ground. Surveys which have limited themselves to a recital of clinical laboratory data (6) or of the animal findings (19) forego any effort to render the whole more comprehensible. It does appear that at least tentatively some coherent use can be made of existing data. Indispensable as the data may be, they constitute less than complete scientific knowledge, and one is tempted to recall, in this connection, the profound observation of Sir Edmund Whittaker—"A common fallacy is to suppose that scientific knowledge is obtained from straightforward observation of phenomena, or from inductive generalizations based directly on experience."

We find it hard to believe that any of the numerous existing adrenocortical steroids is the true, native hormone or that the action of cortisone in the palliation of rheumatoid arthritis is other than pharmacologic. Indeed, it is an overwhelming pharmacodynamic action: the rapid relief of pain, the sudden abatement of muscular spasm permitting flexibility of otherwise frozen joints, the euphoria, and the prompt return to the pre-

[1] Sayers, G.: *Physiol. Rev.* **30**: 241, 1950.

treatment status following cessation of therapy suggest a completely unphysiologic action.

In 1940, some four years after compound E (cortisone) was first isolated from beef adrenal glands and described by Mason, Myers, and Kendall (20), Kendall (21) wrote: "No one compound [of the large number chemically isolated from adrenal tissue] can be regarded as the vital or essential hormone which can produce all the physiologic effects of the gland." In our opinion, these words are just as true today as they were when Kendall wrote them. They imply either that the native secretion is a mixture of principles already known or that it is a single substance not yet recognized. We incline toward the latter belief. It has been a sort of axiom, in the more generally-held view, that the adrenocortical secretion consists of balanced quantities of steroids having the respective predominant actions and, in a measure, complementing or antagonizing the actions of one another. It was on such grounds that Selye (22) and Schaffenberg, Masson and Corcoran (23) were able to postulate an imbalance to explain experimental "arthritis" following desoxycorticosterone.

There is no evidence to show or reason to believe that rheumatoid arthritis results from adrenocortical insufficiency or that treatment with physiologic amounts of cortisone or any other adrenocortical hormone will effect a remission. Why then should one speak of the use of massive doses of cortisone as a "physiologic treatment"?

In the palliation of rheumatoid arthritis with large doses of cortisone alone we believe that the well-known glycogenic action of the compound induces an extraordinary and pathologic synthesis of carbohydrate at the cellular surface-boundary. In this view more carbohydrate is produced by gluconeogenesis than can find use, thus the blood sugar rises and glycogen is deposited in the liver. This performance is carried off at some cost, notably in the loss of cellular protein. But there is a temporary clinical advantage, in that unidentified noxious agents, such as the etiologic factor or factors responsible for rheumatoid arthritis, become unable to provoke inflammation: they are confronted with cells somewhat indestructible for the time being. The disease-producing agent is still present; the change that has been accomplished is to render the cells temporarily less subject to injury and consequently to minimize the associated inflammatory response. One might think metaphorically of these cells as narcotized.

The one hope is that since the cells, benumbed under the influence of cortisone, are able effectively to hide signs and symptoms of the disease, simulating temporary remission, this will somehow develop into a true remission. Insofar as rheumatoid arthritis is concerned there is as yet no evidence that this occurs.

In the present studies we desired to learn whether or not more work could be accomplished by smaller doses of cortisone if, by means of insulin given at the same time, more utilizable carbohydrate material were made available for the action of cortisone upon cellular structure. That is to say, we regard cortisone as 1) inducing the production of new carbohydrate from protein, and 2) incorporating this into the surface-boundary of the general cell. It might appear that insulin subserves the former function at lower cost in physiologic derangement, and enables the latter to proceed further with a given quantity of cortisone. If the curve of the blood sugar is examined from the point of view of the general cell, other things being equal, a drop in blood sugar level signifies carbohydrate removed from the blood and put to use by the cell. Therefore, the effect of administering a quantity of insulin is only superficially reflected by the blood sugar level. The physiologically important effect is to make carbohydrate available to cells without recourse to cellular protein. We believe that the question is answered by the obvious objective and subjective improvement of patients receiving the combination cortisone-insulin.

This is perhaps antithetical in the light of concepts which attribute to 11-oxy steroids a direct antagonism to insulin (19) because of its inhibition of gluconeogenesis induced by the 11-oxy steroids, and because this group of compounds, or at least one of them (24) namely, corticosterone, is able to protect mice against insulin shock. To an extent, if tissue carbohydrate can be made abundant through the interposition of insulin, gluconeogenesis induced by cortisone is an unnecessary or injurious process that can be circumvented with advantage. Far from being an antagonist in human physiology we believe that insulin is a direct synergist to cortisone.

Perera, Pines, Hamilton and Vislocky (25) administered cortisone, 80 mg. daily, for eight days to a hypertensive non-arthritic patient with mild diabetes, "initially controlled by diet alone and later supplemented by small doses of standard insulin." These authors state, "Fasting blood sugar levels were higher during the period of steroid treatment. On the fourth day after the start of compound E the peak levels of blood sugar were higher than in other glucose tolerance tests, and the character of the curve was more abnormal on both tests conducted during the treatment periods. The total daily excretion of reducing substances was markedly increased in association with the drug administration."

Boland and Headley (3) treated one patient with typical rheumatoid arthritis who also had diabetes which was controlled adequately by a measured diet and 10 U. of protamine zinc insulin daily. Twenty-four hours after the initial injection of 200 mg. of cortisone, although still receiving the insulin and measured diabetic diet, glycosuria was noted. "There-

after 30 to 50 U. of insulin were required daily to control the diabetes. Three days after the cortisone withdrawal, insulin reverted to original requirements."

Sprague, Power, Mason and Cluxton (26) reported the treatment of a non-arthritic patient with Addison's disease and diabetes mellitus: "During treatment with compound E there was a marked increase in urinary glucose in spite of the daily administration of four or more times the amount of insulin employed in the control periods."

In commenting on the effect of cortisone on carbohydrate function, Sprague *et al.* (6) concluded that when cortisone is administered in doses of 100 mg. daily to arthritics without diabetes no conspicuous alterations in carbohydrate tolerance were noted. There were, however, some notable individual exceptions. These authors further state that more striking changes might be seen if cortisone were administered to latent or frank diabetics. No data are offered concerning the effect on glucose tolerance of doses higher than 100 mg. per day.

SUMMARY

The daily administration of much smaller doses of cortisone than have been usual in the palliation of rheumatoid arthritis, with insulin, was followed by evidence of improvement in 12 cases, as follows:

Cortisone 12.5 mg., insulin 20 U. None
Cortisone 25 mg., insulin 40 U. Definite progress in 6 of 8 patients.
Cortisone 50 mg., insulin 20–60 U. Major improvement or complete remission in 11 of 12 patients, and minor improvement in 1 (who had irreversible changes that had not previously responded any better to full doses of cortisone for one month).

Excepting infrequent and mild hypoglycemic episodes which were readily controlled in the usual way, side effects did not occur. The period of treatment necessary to demonstrate maximal benefit varied from ten to thirty days. The experience was comparable in all respects, save side actions, with that noted with 100 mg. doses of cortisone (without insulin).

The method is proposed as a field of profitable study, possibly leading to a safer and less expensive palliative management of the disease with cortisone. A number of proposals are offered to account for the action of cortisone, and it is suggested that insulin is supporting to that action by making carbohydrate available without drawing upon protein sources.

In our experience both with cortisone alone and with cortisone plus insulin in the dosages used and for the period of time studied, we have observed striking and unequivocal signs of reversal of the disease process. We have, however, only temporarily obscured the clinical and laboratory evidence of the disease. We refer to our procedure as palliative therapy

because it is just that, and we would insist that, aside from the apparent practical value of such palliation, we have not approached more closely to an understanding of rheumatoid arthritis nor to a means of fundamental control of the disease.

Acknowledgment

The authors with to thank the Management of the Schering Corporation for generously supplying the cortisone and insulin for these studies and for defraying the hospital expenses.

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INDUCTION AND MAINTENANCE OF DECIDUAL CHANGES WITH PROGESTERONE AND ESTROGEN

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THE presence of the corpus luteum during pregnancy led Born, and later Fraenkel (1, 2), to investigate the influence of the corpus luteum on the implantation of the embryo. Ancel and Bouin (3) showed that the corpus luteum induced progestational changes in the endometrium of the rabbit. These findings were confirmed by other workers and climaxed by Corner and Allen (4) with the extraction of an oil-soluble substance from corpora lutea which would induce these progestational changes in the uterus of ovariectomized rabbits. They called the extracted material, progestin, a substance necessary for gestation. Since that time the crystalline hormone, progesterone, has become readily available for clinical use and favorable results have been reported in cases of threatened abortion.

In the early clinical studies, ten to twelve years ago, progesterone was available for use in doses of only 1 to 5 mg. per day. Since that time the supply of progesterone has become so plentiful that doses as high as 100 mg. per day have been used. This increase in dosage has been correlated more closely with the declining cost than with the demonstration of physiologic requirements. Corner and Allen demonstrated the minimal quantities which would bring about progestational changes in the endometrium of the rabbit and also the quantities of progesterone which would maintain pregnancy in the rabbit after ovariectomy (5).

These laboratory findings have also shown that progesterone requires the synergistic action of estrogen to produce the progestational endometrium. Furthermore, different dose ratios of estrogen to progesterone are required in different species to obtain this synergism. The use of these two ovarian hormones has made it possible to induce deciduomata in guinea pigs, rabbits, rats, and mice (6, 7).

There have been no clinical studies to determine whether progesterone and estrogen would induce and maintain decidual changes in the human endometrium. This paper describes the findings obtained in an investigation of this problem.

Received for publication July 15, 1950.

MATERIALS AND METHODS

In order that clinical experimentation might yield the most consistent and reproducible results, we considered the following factors as basic requirements:

1. The women should be free of demonstrable pelvic disease and have normal menstrual periods.
2. They should be in good health and of similar age.
3. They should live in a uniform environment, have similar diets and daily activities.

Institutionalized patients were used, since they fulfill these requirements most conveniently. Ten women, 25 to 35 years of age, with normal menstrual periodicity were chosen, since they should be more uniformly responsive to administered hormone than patients with menstrual abnormalities. In addition to these 10 normal women, 1 subject, aged 35 with a premature menopause and 1 patient with endometrial hyperplasia were also included in the study.

Endometrial biopsies were performed each week, except at the time of a menstrual period. The biopsy specimens were obtained during at least one menstrual cycle before any treatment was given and during the cycle following the cessation of treatment, as control observations in each patient. The 12 women in this study were followed for from four to eleven months and 307 endometrial biopsy specimens were obtained. The hormones were injected intramuscularly and as far as possible the following treatment schedule was completed in each patient:

1. Progesterone,[1] 25 milligrams daily.
2. Progesterone, 25 milligrams and estrogen, 2.5 milligrams (Cyclogesterin[1]) daily.
3. Estrogen (Urestrin[1]), 2 milligrams daily or 5 milligrams daily.

Some patients were also given progesterone, 10 milligrams, and estrogen 1.0 milligram (Cyclogesterin[2]), daily. These courses of treatment were initiated in the premenstrual secretory phases of the cycle and continued until the onset of bleeding, unless stated otherwise.

EXPERIMENTAL

Progesterone plus estrogen

When 25 mg. of progesterone together with 2.5 mg. of estrogen (Cyclogesterin) were given daily, there was a definite delay in the onset of men-

[1] The Progesterone, Cyclogesterin and Urestrin were aqueous suspensions of crystalline hormones.

All hormone preparations were generously furnished by The Upjohn Company.

[2] The 10 mg. doses of Cyclogesterin were in an oil solution.

struation and the endometrial biopsy specimens revealed that a definite decidual reaction was induced.

One woman was kept on treatment for eight weeks; decidual tissue was obtained on four occasions during the first six weeks of treatment (Fig. 1). During the last two weeks, there was a brownish discharge rather than the usual menstrual flow, the decidua was lost, and the endometrium be-

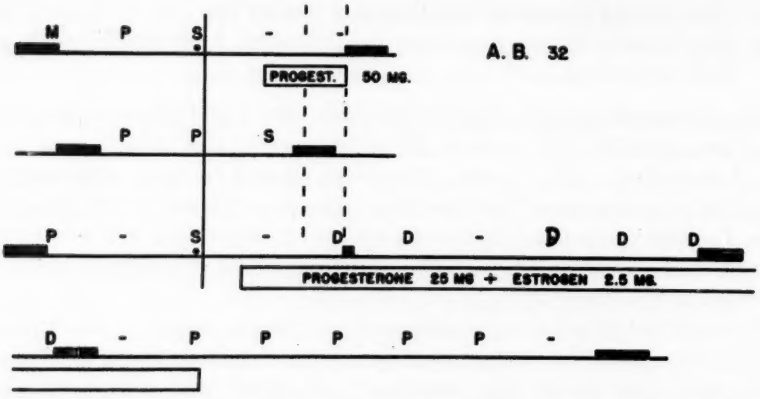


FIG. 1. Patient *A.B.* Progesterone did not delay the onset of the first cycle, but the combined progesterone and estrogen delayed the onset of menstruation for five weeks and a decidual reaction was maintained during this interval. The next cycle was over seven weeks in length and may have been anovulatory but the final premenstrual biopsy specimen was not obtained.

Figures 1 through 5 represent graphically the successive menstrual cycles on several of the study subjects. The solid black bars indicate menstrual periods. The letters denote endometrial biopsies and the regular spacing at seven-day intervals may serve as a time scale. P, indicates a proliferative (estrogenic) endometrium; S, a secretory (progestational) endometrium and D, a decidual reaction. A, denotes an atrophic state and a dash implies that no biopsy specimen was obtained on that day. In each cycle, the first secretory biopsy specimen, usually characterized by subnuclear vacuoles, has been indicated by the small o under the S, arbitrarily placed next to the solid vertical line. On the basis of this early secretory change the next anticipated menstrual period would come ten to fourteen days later, within the vertical broken lines. The dosage and duration of treatment is indicated in the open rectangles under the line representing that menstrual cycle.

came proliferative in appearance. Another subject started to bleed after four weeks of treatment (Fig. 2); the expected menstrual period had been delayed over three weeks and four biopsy specimens had revealed decidual endometrium. The sequence of endometrial changes in these two women is shown in Figures 6–11. The third woman was bleeding after four weeks of therapy; three decidual biopsies had been performed but treatment was

continued for another week. At the end of this fifth week of treatment the biopsy specimen showed an atypical proliferative endometrium. In the fourth patient, bleeding occurred after three weeks of treatment and two biopsy specimens had revealed decidual changes. In two instances (Figs. 3 and 4) treatment was discontinued after the menstrual period had been delayed about one week, but before the onset of bleeding.

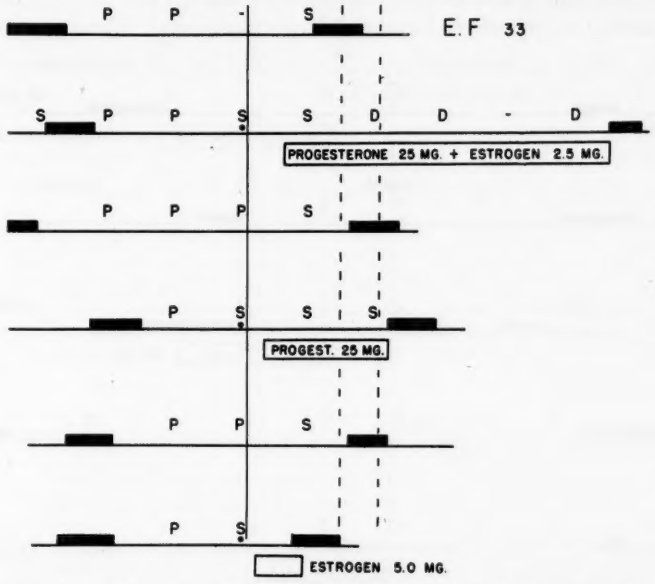


FIG. 2. Patient *E.F.* The combination of progesterone and estrogen delayed the expected menstrual period in the second cycle for over three weeks and a decidual reaction was maintained. The subsequent cycle had a longer proliferative interval and apparently a shortened secretory interval. Progesterone did not delay the next period significantly and estrogen may have shortened the last cycle.

The combined estrogen and progesterone treatment failed to delay the onset of menstruation on three successive trials in one woman, but on the fourth occasion treatment was started earlier in the cycle and the menstrual period was delayed over two weeks and two biopsy specimens of decidua were obtained before treatment was discontinued. It was assumed that probably the first three trials were started so late in the secretory phase that the absorption of progesterone from the crystalline suspension may not have been rapid enough to delay the onset of menstruation.

The patient with the premature menopause (L.A., Fig. 5) was given two weeks of treatment with estrogens (5 mg. of estrone on alternate days)

and then was given 25 mg. of progesterone and 2.5 mg. of estrogen daily for the next three weeks. Decidual changes were not apparent until after two weeks of therapy but were definite by the third week. During the fourth week the dose was doubled (50 mg. of progesterone and 5 mg. of estrogen daily), and the decidua was maintained (Fig. 12). This larger dose was given early in this study before it was found that 25 mg. daily would maintain decidual changes for longer than two weeks.

The patient in whom endometrial hyperplasia was observed, was given

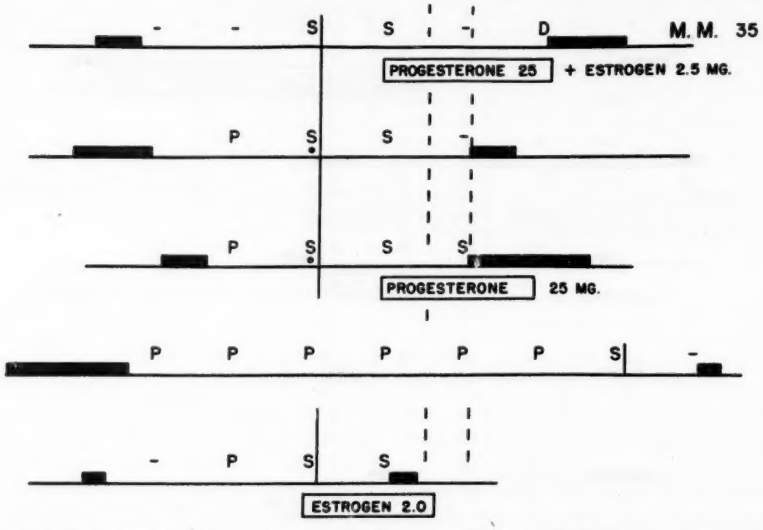


FIG. 3. Patient *M.M.* Progesterone and estrogen had delayed the expected menstrual period about one week and had induced decidual changes at the time treatment was discontinued. Progesterone did not affect the onset of menstruation but the continued treatment may have prolonged the period of bleeding. The recovery cycle was greatly prolonged and the last cycle may have been shortened by the administration of estrogen.

progesterone and estrogen (25 mg. plus 2.5 mg.) daily, starting nearly five weeks after her previous period (K.D., Fig. 5). Secretory changes were noted in the first two weeks of treatment but decidual changes were not evident until the third week (Fig. 13) and then she menstruated in spite of continued therapy.

Smaller doses of progesterone and estrogen were also tried (Figs. 4 and 5). Five women were given a daily dose of 10 mg. of progesterone and 1 mg. of estrogen but they did not experience any delay in expected menstruation, nor any decidual formation.

Progesterone

In contrast to the demonstration that progesterone plus estrogen would induce and maintain a decidual reaction for from two to five weeks in the experiments just described, was the consistent failure of progesterone alone to postpone the onset of the expected menstrual period or induce a full decidual change. In only 1 instance did the biopsy specimen show an endometrium which was decidual in character (G.W.) but menstruation was

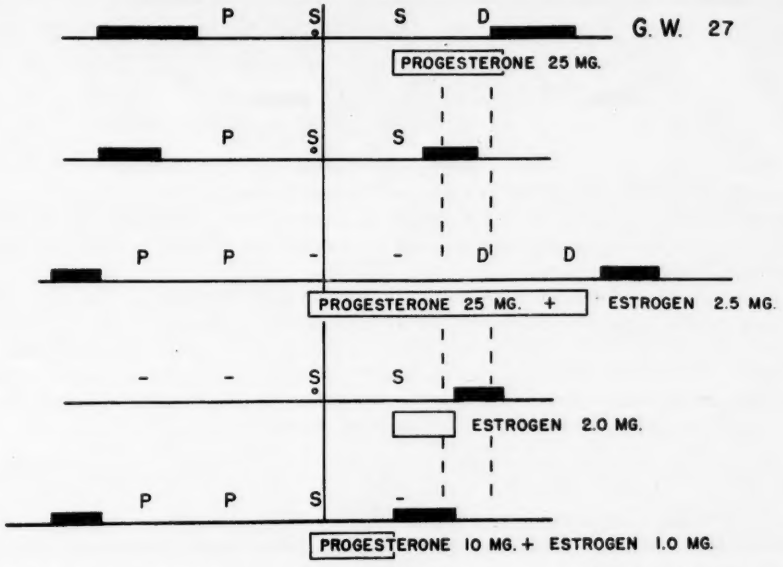


FIG. 4. Patient *G.W.* Progesterone did not delay the onset of menstruation or affect the length of the subsequent cycle. Progesterone and estrogen had delayed the expected period over a week and had induced decidua formation when treatment was discontinued, to allow the patient to go home for a month. The patient returned to the hospital in the early secretory phase and a course of estrogen treatment did not affect the the onset of the expected menstrual period. The low dose of progesterone and estrogen in the last cycle may have hastened the onset of bleeding.

not delayed (Figs. 1, 2, 3, and 4). In 6 women, menstrual bleeding started during progesterone treatment and usually after only six or seven days of treatment.

The longest period of progesterone treatment was twelve days (Fig. 2). This prolongation was due to the fact that therapy was started so early in the secretory phase. In several instances the progesterone treatment was continued for several days after the onset of bleeding. These findings show

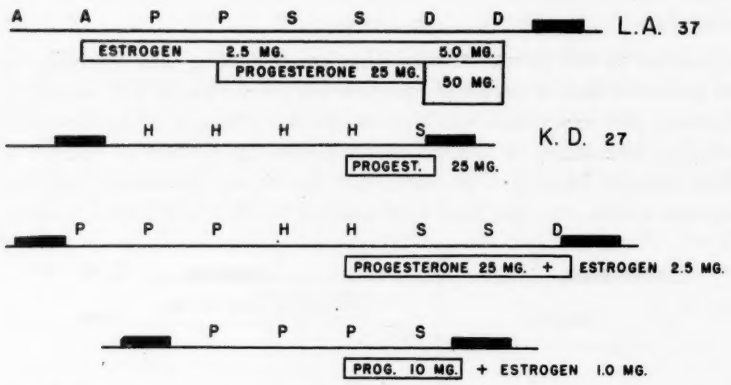

A A P P S S D D
L.A. 37
ESTROGEN 2.5 MG.
5.0 MG.
PROGESTERONE 25 MG.
50 MG.
H H H H S
K. D. 27
PROGEST. 25 MG.
P P P H H S S D
PROGESTERONE 25 MG. + ESTROGEN 2.5 MG.
P P P S
PROG. 10 MG. + ESTROGEN 1.0 MG.

FIG. 5. Patient *L.A.* Premature menopause. The atrophic endometrium was brought into an active proliferative phase by a two-week course of estrogen. Progesterone therapy then produced progressive secretory changes in the next two weeks and then decidua was present in the third and fourth weeks of treatment. Menstruation started four days after cessation of treatment.

Patient *K.D.* Endometrial hyperplasia. In the first cycle progesterone converted the endometrium to a secretory state but did not maintain it. In the second cycle the combined progesterone and estrogen produced and maintained the secretory changes until a decidual change occurred before the onset of bleeding. The lower dose of progesterone (10 mg.) and estrogen also induced early secretory changes but was not sufficient to produce a full premenstrual condition before bleeding started.

FIG. 6. Control biopsy specimen. Early secretory phase characterized by subnuclear vacuoles in the glandular epithelium. *See* S in second cycle in Figure 2. (The magnification in Figures 6–13: ×280.)

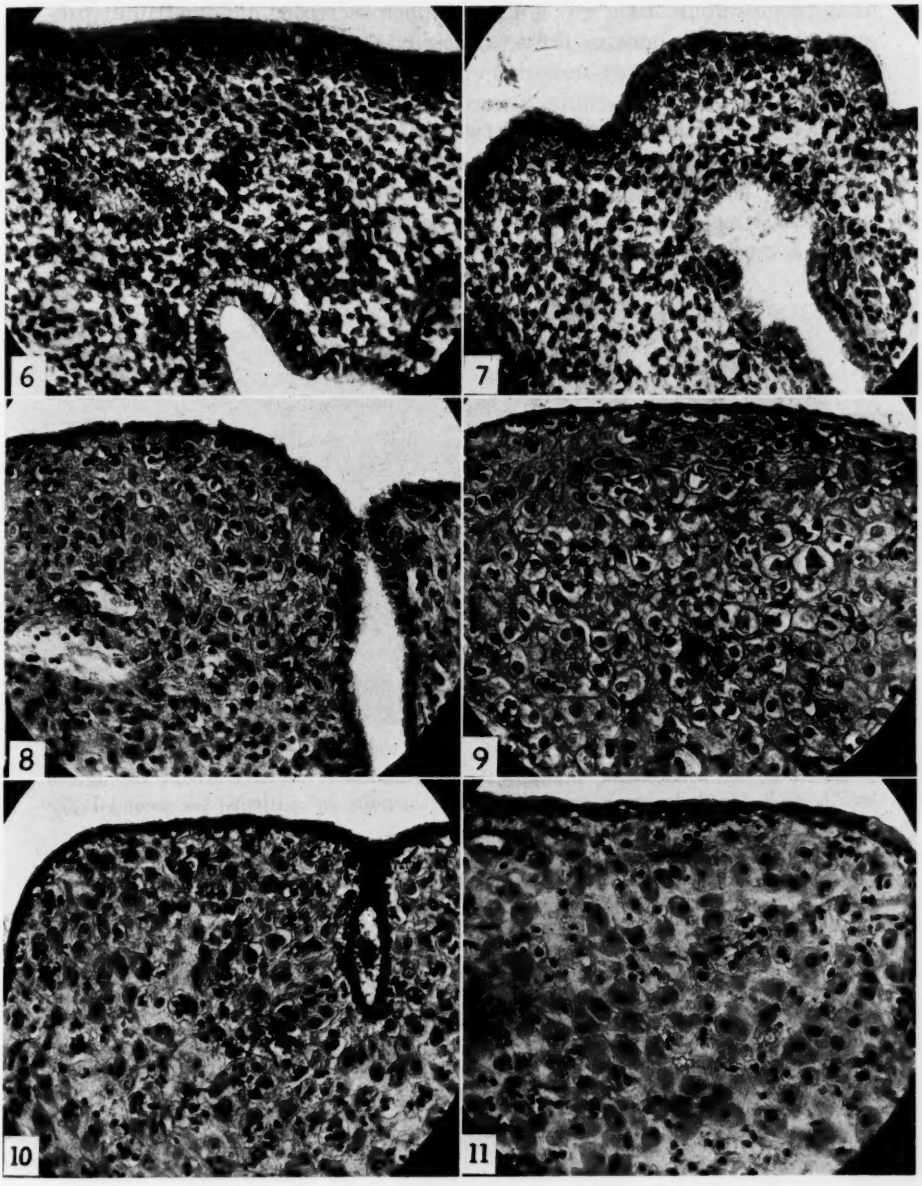
FIG. 7. Biopsy specimen obtained after two days of treatment with progesterone and estrogen (25 mg. plus 2.5 mg.). Normal premenstrual endometrium; glandular epithelium with basal nuclei and frayed border. Edematous stroma. Too soon for any effects of treatment to be evident. *See* S in second cycle in Figure 2.

FIG. 8. Biopsy specimen obtained after nine days of treatment with progesterone and estrogen (25 mg. plus 2.5 mg.). Decidual changes are evident in the stroma and there is an increased vascularity. Low cuboidal epithelium. *See* D between vertical dashed lines in Figure 2.

FIG. 9. Biopsy specimen obtained after thirty days of treatment with progesterone and estrogen (25 mg. plus 2.5 mg.). Decidual changes in stroma and very low pavement type surface epithelium. *See* third D in Figure 2.

FIG. 10. Biopsy specimen obtained after thirty days of treatment with progesterone and estrogen (25 mg. plus 2.5 mg.). Similar to specimen in Figure 9. *See* third D in Figure 1.

FIG. 11. Biopsy specimen obtained after forty-four days of treatment with progesterone and estrogen (25 mg. plus 2.5 mg.). Cell membranes not so distinct and some nuclei have lost their chromaticity. Early stages of dissolution. *See* fifth D in Figure 1.



FIGS. 6–11

that progesterone alone will not maintain a secretory endometrium (prevent menstruation) or convert it to decidua.

Estrogen

Since the obvious difference between the two contrasting experiments above is that estrogen was used in one and not in the other, it was deemed necessary to try estrogen alone. Seven trials were made, in which 2 mg. of estrogen (Urestrin) was injected daily, starting in the luteal phase of the cycle. A daily dose of 5 mg. was given in 3 instances. In no case was the

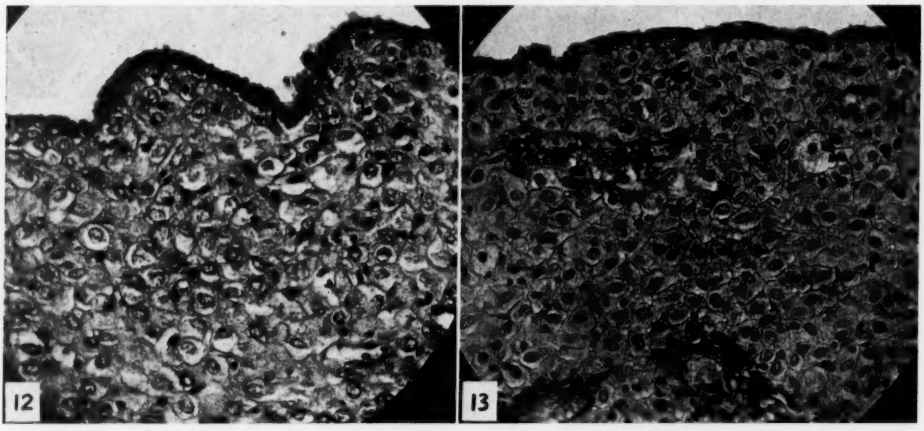


FIG. 12. Biopsy specimen obtained after four weeks of treatment—three weeks of progesterone and estrogen (25 mg. plus 2.5 mg.) and then double this dose during the fourth week. Good decidual reaction in a postmenopausal patient. *See* second D for L.A. in Figure 5.

FIG. 13. Biopsy specimen obtained after three weeks of treatment with progesterone and estrogen (25 mg. plus 2.5 mg.). Definite decidual change; but this was not maintained as long as in the other patients. *See* D in second cycle of K.D. in Figure 5.

onset of menstruation delayed by estrogen but in several cases it seemed to be induced prematurely. In no case was any decidual reaction found in the endometrium.

These findings indicate that estrogen is necessary to augment the effect of the progesterone but that neither substance is capable of inducing or maintaining a decidual change when given independently.

DISCUSSION

To the authors' knowledge this is the first study which has attempted to determine quantities of estrogen and progesterone which would induce

decidual change in normal women. Szarka (8) induced decidual changes in an ovariectomized woman. He used estradiol benzoate in doses of 5 mg. daily for a month, then gave 10 mg. of progesterone daily for one week, followed by 20 mg. doses of progesterone in addition to the estrogen in the second month of treatment. The estrogen dose was discontinued during the fourth week and even though the progesterone was continued, the woman started to bleed and passed a decidual cast. His observations are similar to ours, in that the progesterone dosage was comparable and that it was not effective after the estrogen was discontinued. There were no endometrial biopsies during the period of treatment.

The only study in which endometrial biopsy specimens were obtained at regular intervals, showing the progressive histologic changes leading up to early decidual changes, also utilized ovariectomized women (9). Progesterone was not given longer than two weeks in any of those observations so that full decidual changes were not obtained and the proportion of estrogen was too low in some instances.

The foregoing findings emphasize the well known synergistic action of estrogen and progesterone. They also indicate that the ovary, and probably the corpus luteum itself, produces appreciable quantities of estrogen in addition to progesterone during the luteal phase (10). In experimental animals it has been found that when estrogen exceeds a ratio of more than 1 to 1000, it tends to nullify the action of progesterone (11, 12). The high ratio of estrogen to progesterone (1 to 10) used in the present study, shows that estrogen does not nullify the effects of progesterone in women as it does in animals.

Szarka had a ratio of 1 to 4 in his experiment. The fact that stilbestrol has been used in doses of 25 mg. per day in cases of threatened abortion, indicates that even in ratios that approach or exceed 1 to 1, estrogen still may not hinder the progesterone effects in women. Further studies should be made to determine the optimum ratio of estrogen to progesterone.

The failure of estrogen to delay the onset of menstruation (its failure to maintain the corpus luteum when treatment is started in the luteal phase) is consistent with earlier reports in women (13). The failure of progesterone alone to delay the onset of menstruation is also compatible with the earlier reports of the use of progesterone in the treatment of dysmenorrhea. The onset of bleeding during treatment, especially with progesterone alone, shows that it has little, if any, immediate hemostatic effect. This is called "break-through" bleeding by Willard Allen and co-workers (14). The vascular nature of the decidua which was formed in these experiments suggests the possibility of rather profuse hemorrhage at the end of treatment. However, those patients experienced shorter and generally less profuse menstrual periods than usual after the decidual changes were induced.

In all probability, the corpus luteum regressed during the period of progesterone-estrogen treatment and the decidual changes were induced and maintained by the injected hormones. Certainly the findings were duplicated in L. A. (the woman with the premature menopause), in whom there was no ovarian participation even in the preparatory phases of the treatment. At least there is no reason to assume that the treatment maintained the corpora lutea which were present initially. It is anticipated that if a patient is subjected to laparotomy for the excision of a fibromyoma after several weeks of progesterone and estrogen therapy, it should be possible to demonstrate the involuted corpus luteum.

These studies may not indicate the amounts of hormones produced by the corpus luteum in early pregnancy. They do, however, indicate that quantities approaching 25 mg. per day of progesterone, together with adequate estrogen, will induce decidual changes comparable to those of early pregnancy. Further study is necessary to determine whether larger doses will maintain the decidua longer than the four to six-week intervals obtained in this study. It is hoped that ultimately the daily physiologic requirements may be determined, so that replacement therapy in threatened abortion may be physiologic rather than empiric.

SUMMARY

When started during the luteal phase of a normal menstrual cycle (10 cases), daily doses of 25 mg. of progesterone combined with 2.5 mg. of estrogen delayed the onset of the expected menstrual period from three to six weeks and induced and maintained decidual changes in the endometrium. Smaller doses (10 mg. of progesterone and 1 mg. of estrogen) did not have this effect.

When 2 women with abnormal ovarian function were given adequate preliminary estrogen, daily doses of 25 mg. of progesterone combined with 2.5 mg. of estrogen induced the normal sequence of secretory changes in the endometrium during the first two weeks of treatment; then the decidual changes became evident during the third week of treatment. The time sequence and the histologic changes were similar to those seen in the endometrium of the women with normal cycles.

Daily doses of 25 mg. of progesterone or daily doses of 2 to 5 mg. of estrogen given to women with normal cycles did not delay the onset of an expected menstrual period or induce any decidual changes in the endometrium.

Further studies are necessary to determine the optimal proportions of progesterone and estrogen to obtain maximal synergism between these two hormones.

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THE EFFECT OF STEROID HORMONES IN RHEUMATIC HEART DISEASE

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THE remarkable association between pregnancy and remissions of rheumatoid arthritis has long been recognized, and has repeatedly been the subject of comment; it has even been a pretext for administering various hormones not known to be related to pregnancy. The effort to supply therapeutically the steroids of pregnancy, in amounts comparable with those actually present normally, has not previously been reported. The present report describes the result of such treatment in active rheumatic fever, with polyarthritis or carditis, or both, in 64 patients, using massive dosage of progesterone and estradiol.

MATERIAL AND METHODS

We have treated 64 patients with rheumatic fever in the manner described (1), during the period 1946–1950. The ages of these patients ranged from 3 to 39 years at the time treatment was begun. There were 20 patients with typical polyarthritis, 10 with rheumatic carditis, and 34 with both features; and an additional 16 patients with chorea will be reported separately.

The disease was evaluated by the standard criteria, before, during and after treatment, and during the period of follow-up, which in some cases is now as long as four years. Determinations included physical findings relating to the heart, the affected joints, and the general examination; erythrocytic sedimentation rate; white cell count, red count and hemoglobin; and x-ray cardiac silhouette.

Treatment consisted of single, simultaneous intramuscular injections of 500 mg. of progesterone and 33.3 mg. of estradiol benzoate,[1] the two steroid solutions (in oil) being mixed in one syringe and half the material injected into each buttock. The intervals between doses were four to eight days. For children younger than 15 years, one-half this dosage or less was employed.

The dosage is notably higher than is usually employed for the treatment of any condition of which we are aware. In order to emphasize the magnitude of dosage, and its importance, we draw attention to the various determinations of steroid hormone excretion during later pregnancy, from

Received for publication July 10, 1950.

[1] Estradiol benzoate as Progynon-B and progesterone as Proluton were kindly provided by the Schering Corporation, Bloomfield, N. J.

which it can be calculated that the endogenous production of these hormones is of an extremely high order. The rationale of treatment was to approach those magnitudes.

Thus, 500 mg. of progesterone represents 500 international units or approximately an average daily secretion in late human pregnancy as computed from the output of sodium pregnandiol glucuronidate. The dosage of estradiol benzoate, 33.3 mg. or 200,000 rat units, corresponds to 2,000,000 international units, and is assumed to be at least as large as the high values encountered in normal pregnancy, although there are difficulties in concluding even approximately how large these actually are.

Appraisal. In order to rule out the simultaneous action of other treatment, such as that used for symptomatic relief in these patients, we established a pretreatment control period of three or four days during which only codeine was given, as required for pain; but with the first hormone dose, even this was discontinued. We define three grades of response: 1) *satisfactory*, in which all signs of rheumatic activity disappeared, although cardiac murmurs may of course have persisted; 2) *slight*, in which pain and temperature were reduced, but not abolished, and other signs remained—the symptoms usually being found to progress unfavorably after hormone treatment was discontinued; and 3) *none*, in which no benefit could be identified in connection with the administration of hormones.

RESULTS

Of 64 patients treated with steroid hormones in this way, 4 went on to die of rheumatic heart disease or its complications (1 of pancarditis, and 3 in congestive failure). Of these 4, 3 had previously responded to some extent to treatment with hormones, but it cannot be ascertained whether or not reactivation of the disease had been more delayed as a result. On the contrary, there is a growing clinical impression that recrudescences of rheumatic fever have become less frequent in general, since sulfonamides and antibiotics have come into widespread use in respiratory infections. Therefore a detailed statistical appraisal of our present results would not be appropriate.

Our results are summarized in the following table:

Diagnosis	Results of treatment		
	Satisfactory	Slight	None
Rheumatic fever	17	1	2
Rheumatic carditis	7	1	2
Rheumatic fever and carditis	25	3	6
Totals	*49*	*5*	*10*

The responsive patients exhibit a distinctive pattern. Within three to eight hours after the injection, there is symptomatic and objective improvement in the inflamed joints, and all arthritic activity disappears within forty-eight hours. The temperature, if very high, usually falls precipitously, reminding one of the crisis in lobar pneumonia. Indeed, the more acute the rheumatic attack, the more rapid and dramatic we have found the response to treatment. Patients have commonly demanded to be allowed up twenty-four hours after the first dose. Our procedure has been to repeat the injection when there is a rising temperature, increased heart rate, or other sign of recrudescence; the effect of subsequent doses is less dramatic but more lasting when compared with the initial dose in a given patient. A favorable result has been sustained, in general, by repeating the dosage at intervals of four to eight days. From two to nine injections have been needed during any one attack.

Illustrative Cases

V. P. was a 10-year-old girl who was admitted to St. Francis Hospital because of the finding of an elevated sedimentation rate during her visit to the Cardio-Rheumatic Clinic. Two years before she had had an appendectomy which was followed in one month by chorea plus mildly active polyarthritis. This necessitated hospitalization for five weeks. She remained well for eleven months; then chorea and arthritis recurred, resulting in another stay in the hospital for six weeks and in a convalescent home for several months. With complete inactivity she returned home.

On admission the temperature was 100, apex rate 94 and respirations 20. She was pale but had no complaint. She was a well-developed and well-nourished girl with a grade 1 mitral systolic murmur. The sedimentation rate was 44 mm. in one hour (Landau). The hemoglobin was 81 per cent, red blood cells 4,000,000 and white blood cells 8,600 with a normal differential count. The electrocardiogram was entirely normal with a P-R interval of 0.16 second. During the first five days in the hospital no treatment was given. On the sixth day she became explosively and critically ill with polyarthritis, pancarditis, gallop rhythm and severe congestive failure. The temperature rose to 105, apex heart rate to 160, and the respiratory rate to 80. The characteristic findings of pericarditis and myocarditis were present in the ECG tracings.

She was given 250 mg. of progesterone and 16.67 mg. of estradiol intramuscularly, which produced a state of euphoria but no other change in the clinical condition. Twenty-one hours later the same dose was repeated. After eleven hours the temperature dropped to normal, she was free of all joint pains, and she was sitting up in bed reading a comic book and begging to be allowed out of bed. From then on, there was gradual improvement following four additional hormone injections at six-day intervals. She was afebrile from the thirty-eighth day, all signs of congestive heart failure were absent, the ECG had returned to normal and the heart itself revealed a short grade 1 mitral systolic murmur. The sedimentation rate did not become normal until the sixtieth day. (Three nationally recognized cardiologists and pediatricians saw this patient at my request and all stated that it was their opinion that the hormones had a major part in saving the life of this girl.)

M. C. was a 14-year-old boy who had frequent nose-bleeds for a year before his present illness. Ten weeks prior to admission to the hospital he was confined to bed at home with typical rheumatic polyarthritis. His course at home was unsatisfactory. The day before entrance the attending physician detected clinical signs of pancarditis and advised hospitalization.

On admission the temperature was 103, pulse 130, respiration 30, and sedimentation rate (Westergren) 67 mm. in 1 hour. He was pale and perspiring profusely. Both ankles, knees, elbows and shoulders were red, swollen and painful. His heart was enlarged to the left; a pericardial friction rub was present and both sounds were of poor quality with a grade 5 mitral systolic murmur. The lungs were clear. B.P. was 100/60. For the first three days he was given small doses of codeine for comfort but on the fourth day he was extremely ill; he had a sedimentation rate of 114 mm. and a persisting P-R interval of .24 second. He was given 500 mg. of progesterone and 33 mg. (2,000,000 International Units) of estradiol intramuscularly. Four hours later his joints had improved and he felt generally better. In twenty-four hours all joint pains had disappeared, temperature had dropped two degrees and the patient was insistent on getting out of bed. Forty-eight hours after therapy the temperature was normal, the pericardial friction rub was not present and the pulse was 90. On the eighth day following the treatment the sedimentation rate had reached 84 mm. but a rise in temperature after six days of no fever prompted a second injection of both massive doses of progesterone and estradiol. Two days later the P-R interval was .16 second. He remained afebrile, symptomless and ceased his perspiring for the rest of his hospital stay which required thirty days for the sedimentation rate to reach normal. The heart sounds improved in quality but a grade 4 mitral systolic murmur was residual. He was discharged on the forty-ninth day, receiving daily doses of sulfadiazine, and has had no recurrences in almost four years.

A. M. was a 13-year-old girl who had smouldering rheumatic fever at home for eleven months. On admission to the hospital her temperature was 100.8, pulse 136 and respiration 22. The sedimentation rate was 124 mm. in 1 hour. She complained of pain in her ankles and knees. Her heart was normal and the P-R interval was .15 second. After a pretreatment control period of four days she was given the full massive doses of 500 mg. of progesterone and 33 mg. (2,000,000 International Units) of estradiol, since she was physically much older than her years. There was no change in either her clinical or laboratory findings and the same dose of hormones was repeated on the sixth day. The joints became worse and the sedimentation rate rose to 136 mm. She was so uncomfortable that large doses of codeine were required to obtain relief. After an interval of two weeks when she appeared comparable to the time of her entrance to the hospital, she was treated again with 250 mg. of progesterone and 16.67 mg. of estradiol without any effect. Her family refused to keep her longer in the hospital or to permit any additional injections.

R. LeG. was a 3-year-old girl who had active rheumatic polyarthritis, pancarditis, gallop rhythm and congestive heart failure. She had been receiving salicylates, penicillin and oxygen for a period of two weeks before she was placed on the Cardio-Rheumatic Service. Examination by this Service revealed a very sick child with a temperature of 104, apex rate of 160, and respiration 66. The sedimentation rate was 55 mm. in 1 hour (Landau). Her heart was enlarged, the sounds were of poor quality with gallop rhythm, and both systolic and diastolic murmurs present. Both lung bases showed rales and the liver was enlarged to the iliac crest. The ECG manifested typical signs of pericarditis and myocarditis.

She was immediately given 125 mg. of progesterone and 8.3 mg. (500,000 International Units) of estradiol, with marked improvement in forty-eight hours. Four days later the dose was repeated with further subsidence of the temperature which reached normal ten days after the first treatment. The sedimentation rate dropped to 41 mm., but the temperature remained normal for only forty-eight hours. A third dose of the hormones brought the temperature to normal again for only forty-eight hours. Thinking that the dose might be slightly increased in order to obtain a more lasting effect, 175 mg. of progesterone and 11.7 mg. (700,000 International Units) of estradiol were administered. No additional effect was noted and it was deemed advisable not to increase the dose further. She received seven injections over a six-week period and although the clinical heart findings and the ECG patterns gradually returned to normal during this time the temperature and sedimentation rates did not. Six days following her last hormone treatment she had very scant but definite uterine spotting which persisted for three days. It did not recur. (This patient illustrates a "slight response.")

Side actions. As must be expected, the introduction of active steroids in high dosage produces a number of side effects which are not wholly desirable; but with the hormones we have used, they were minimal and temporary, and consisted of exaggerations of normal physiologic phenomena.

Among them were euphoria, drowsiness, a small degree of mammary enlargement in either sex, and uterine withdrawal-bleeding. In some older males there was a slight diminution in libido.

Euphoria has not been objectionable, but since it is expressed often as a desire of the patient to be up and about, when the cardiac status may not justify this, the patient's inclination must be disregarded and necessary bed rest continued.

The sense of drowsiness occurs shortly after the steroid dose, ordinarily within thirty to sixty minutes, and seems to last for four to six hours. It is possible that in this range of dosage we approach that "anesthetic" action of steroids which has been described after immense quantities of steroid hormones in the rat and in fish. It is, however, far from anesthesia, and is transitory in any event.

Scant uterine bleeding occurs in all females of reproductive age, eight days after the single dose of progesterone-estradiol; or, if the dosage is repeated at intervals of less than six days, it is apt to follow the last injection of the series. It has not been excessive. In prepubertal girls also, a small amount of bleeding may occur, but is not serious; nor are other harmful effects observed in these younger patients, concerning whom we were especially watchful.

DISCUSSION

The mode of action of the steroid hormones in the rheumatic state is not understood; this state of affairs prevails for gonadal steroids quite as much as for adrenal compounds. As often mentioned, rheumatic and rheumatoid activity are at a minimum during pregnancy; and although

pregnancy is a very serious complication in marginally compensated rheumatic heart disease, it is the additional cardiac work, leading to failure, more than reactivation of the basic disease, which is apt to cause trouble.

It is of further interest here, even though it does not go far to clarify the problem, that in our experience the combination of progesterone and estradiol in this dosage range has been capable of duplicating the effects of cortisone (or of ACTH) in the so-called collagen diseases other than lupus erythematosus; and even in some of the heterogeneous disorders (*e.g.*, peptic ulcer) in which massive doses of adrenocortical steroids are temporarily effective. One may speculate whether these gonadal hormones exert a marked "central" action leading to the production of ACTH, or whether they act in the tissues themselves, as the influence of pregnancy rather suggests. Our continuing studies are calculated to aid in clarifying this.

SUMMARY

Of 64 patients with rheumatic fever who were treated with massive single doses of progesterone and estradiol benzoate, such as 500 mg. and 33 mg., respectively, at intervals of a few days, 49 patients or 77 per cent showed a response consisting of a temporary arrest of all activity; and 5 patients or 8 per cent showed an incomplete response, but with definite immediate evidence of improvement. To a considerable extent, the clinical effects which have been reported to follow massive dosage of cortisone or of ACTH can be duplicated by the combination of progesterone and estradiol in massive dosage and the procedure, in our experience, has not been attended by overwhelming metabolic derangements. The plan of treatment is based on the high steroid hormone levels present during pregnancy and is calculated to reproduce these levels, within practical limits, in order to bring about rheumatic remissions in the manner characteristic of pregnancy. The results are submitted for confirmation, and further studies are under way to account for the mechanisms concerned.

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ADRENAL CORTICAL METABOLISM IN CHRONIC LIVER DISEASE

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AN EVALUATION of adrenal cortical function in patients with chronic hepatic disease and fluid retention was conducted in the light of the established role of this gland in salt and water metabolism. In addition, one group of patients with liver disease was studied in special detail because certain clinical features suggested a particular functional derangement of the adrenal gland. Adrenal and adrenocorticotropic[1] hormones were administered in the course of the study, although the use of the latter was limited by its toxic effects.

The subjects studied were patients at the Hospital of The Rockefeller Institute. All had evidence of moderate or advanced hepatic cirrhosis with or without ascites. They were classified into three groups: 1) so-called Laennec's cirrhosis; 2) biliary cirrhosis of the xanthomatous type; 3) cirrhosis of undetermined etiology confined to young females, described more fully later. Some patients were included from the group reported by Eisenmenger *et al.* (1) who were on rigid dietary sodium restriction.

METHODS

The determination of urinary 17-ketosteroids was made by means of the Zimmermann reaction in which the modification of Drekter (2) and Kenigsberg *et al.* (3) was used. Twenty-four-hour specimens of urine were collected and the appropriate aliquot examined. The 17-ketosteroid excretion was determined several times weekly on patients included in the study. Girard's reagent was employed for the fractionation of 17-ketosteroids into ketonic and nonketonic components in a few instances, with digitonin precipitation for separation into alpha and beta compounds. The release of 17-ketosteroids by hydrolysis with bacterial glucuronidase, according to Buehler and coworkers (4), was investigated in a number of patients.

Adrenal "corticoids" in the urine were measured for the most part as neutral lipid-reducing compounds by the method of Heard and Sobel

Received for publication July 13, 1950.

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[1] Adrenocorticotropic hormone was provided through the courtesy of Dr. John R. Mote of The Armour Laboratories, Chicago, Ill.

(5, 6). These determinations were performed frequently and at short intervals in the course of illness and after institution of therapy. Several specimens of urine were examined for corticoids liberating formaldehyde on periodic oxidation as described by Daughaday, Jaffe and Williams (7). This method was modified by the omission of benzene-water partitioning. Romanoff, Plager and Pincus (8), by studying diurnal variations, have shown a close correlation between formaldehyde-generating and copper or phosphomolybdic-reducing steroids in urine, although the absolute values were different. In addition, they indicated that the phosphomolybdic acid reduction was least variable and less cumbersome than other techniques. For these reasons it was used for routine study in the course of these investigations. Glycogen-depositing steroids excreted in the urine were studied by the Venning bioassay technique (9) in a representative group. Finally, the bioassay method of Deming and Luetscher (10) for the demonstration of desoxycorticosterone-like action of urine steroids was utilized.

Serum albumin was determined by the sodium sulfate precipitation method modified to obtain an accuracy of ± 3 per cent. Albumin was further checked by means of the immunochemical technique of Kunkel and Ward (11) in which the ninhydrin reaction is used, and by means of electrophoretic patterns. Globulins were studied by means of the zinc turbidity test (12), electrophoretic and immunologic techniques.

Blood sugar levels were measured according to the method of Somogyi-Shaffer-Hartman (13); creatine and creatinine were determined by the use of the picric acid procedure as modified by Clark and Thompson (14).

Sodium and potassium in biologic fluids were measured by use of the Perkin-Elmer flame photometer with lithium as an internal standard. Samples of sweat were collected from selected areas of the patients during exposure to heat applied to the entire trunk by means of electric cradles. Saliva samples were obtained in the morning before any activity.

Eosinophil counts were performed on venous blood; the eosin-acetone-aqueous solution of Dunger as outlined by Thorn *et al.* (15) was utilized.

Total serum lipids were determined according to the manometric lipid carbon method of Ahrens, Eder and Van Slyke (16). Lipid phosphorus was measured by means of a modification of the technique of Fiske and Subba-Row (17) and converted to phospholipid by the factor 25. The Schoenheimer-Sperry (18) method was used for cholesterol determinations.

EXPERIMENTAL

The patients were divided into three groups as indicated above. Those with Laennec's cirrhosis had ascites and marked sodium retention, with the exception of A. L., who was convalescent and was exhibiting sodium

and water diuresis following prolonged maintenance on dietary sodium restriction. These patients uniformly had a background of poor nutrition associated in many instances with the imbibition of excessive alcohol over a long period. The patients with biliary cirrhosis comprising the second group had long histories of painless jaundice without operative findings of extrahepatic obstruction. Evidences of portal obstruction appeared only as late manifestations. The third group of patients with an unusual cirrhosis of undetermined etiology has been of particular interest. This type of cirrhosis has been confined to females, usually under 35 years of age. Jaundice was of insidious onset and varied in degree over a prolonged period. Many exhibited hirsutism, acne, abdominal striae (without previous ascites), obesity, amenorrhea, and "moon facies." These features appeared concomitantly with the onset of jaundice or shortly thereafter; they did not appear to be sequelae of prolonged hepatic disease but were associated with the earliest manifestations of the illness. When first seen, these patients complained only of jaundice and weakness. Careful interrogation relative to the onset revealed none of the gastro-intestinal symptoms typical of infectious hepatitis, nor was exposure to possible causes or other cases of hepatitis elicited. The Cushing-disease-like findings were uniformly present at the time of the first examination and in some patients antedated the appearance of icterus. These signs, with the exception of amenorrhea, have not been observed in other types of liver disease. However, a direct secondary relationship to hepatic malfunction cannot definitely be excluded at the present time. It is too early in this experience to define the outcome. The jaundice is continuous or recurrent, however, and in several of the patients advanced cirrhosis has ultimately developed. The most characteristic laboratory finding in this last group has been the elevated serum protein and globulin, the latter as high as 10.0 grams per cent or more in several instances. Only a portion of this group under observation has been included in the present study. Chromatographic steroid analyses of urines of these patients are presently being conducted. These together with a more complete clinical picture are to be the subject of a subsequent report.

The frequent occurrence of ascites with renal salt and water retention in cirrhosis of various types is well known. The many factors which contribute to the retention of salt and water include portal hypertension, excessive pituitary antidiuretic hormones, and hypoproteinemia. The established relationship of the adrenal cortical hormones to the regulation of salt and water metabolism stimulated an investigation of adrenal cortical function in hepatic cirrhosis. Furthermore the observed clinical signs resembling those of Cushing's syndrome, which we have delineated as of "undetermined etiology" strongly suggest involvement of the adrenal cortex.

RESULTS

The urinary excretion of 17-ketosteroids

The 24-hour 17-ketosteroid excretions are shown in Table 1 and are based on daily determinations before treatment. Because of the variations with age and sex, the normal value in each instance is given. These values were compiled from our data and those of Kenigsberg (3) who used the same method for the determination of these substances. The values were

TABLE 1. 17-KETOSTEROID EXCRETION IN CIRRHOSIS

	Pt.	Sex and age	Daily excretion		Normal avg. for age-sex
			Range	Avg.	
			mg.	mg.	mg.
Laennec's cirrhosis	AS	M 59	3.5–11.1	7.2	9.0
	VV	M 64	3.1– 6.9	5.0	9.0
	AL	M 56	8.8–20.0	12.3	9.0
	WJ	M 59	3.4–10.7	6.6	9.0
	MR	F 45	3.6– 9.6	6.8	9.3
	LS	F 48	6.1	6.1	9.3
	ND	M 62	4.4–10.2	7.6	9.0
	RM	M 57	4.6–10.2	6.8	9.0
Undetermined cirrhosis	CG	F 19	5.7–13.2	9.6	10.2
	MS	F 16	7.0–11.5	9.2	9.0
	DM	F 19	3.9– 7.0	5.3	10.2
	LB	F 50	4.0	4.0	8.6
	BH	F 38	6.9– 7.4	7.1	9.0
Biliary cirrhosis	MH	F 46	3.5– 9.4	7.0	9.3
	LG	F 50	5.1	5.1	8.6
	MP	F 46	9.4–13.2	10.7	8.6

generally depressed, especially in the group with Laennec's cirrhosis. A. L. was an exception, well on the way to recovery, no longer retaining sodium and water and in an improved nutritional state. There was a relatively wide scattering of figures for each patient and this has been related to urine volume. Those patients who generally retained fluid showed marked variations in urine volume from time to time. This relationship between urine volume and 17-ketosteroid excretion is charted in Figure 1.

The ketosteroids were fractionated in a few instances into nonketonic and alpha- and beta-ketonic steroids (Table 2). The decline in 17-ketosteroids in Laennec's cirrhosis was found mostly in the alpha fraction. Hamil-

ton (19) has shown that the greatest diminutions with age occur in this fraction. The alpha-steroids were lower in 2 of 3 patients than the normal for their age according to him. In the 1 patient with cirrhosis studied following ACTH therapy, the rise in total 17-ketosteroids was almost entirely due to an increase in alpha-steroids.

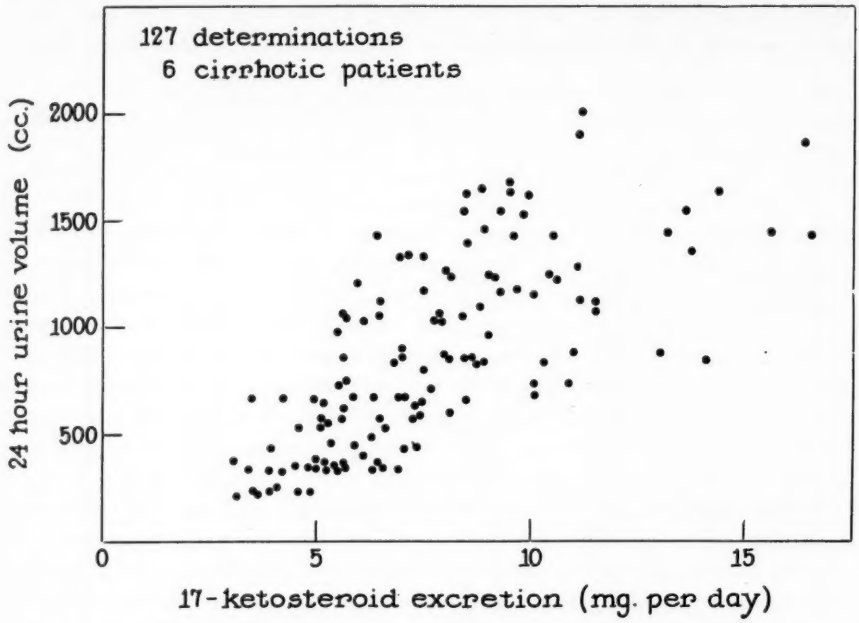

FIG. 1. The total daily 17-ketosteroid excretion plotted against urine volume from multiple determinations in 6 patients with Laennec's cirrhosis.

TABLE 2. 17-KETOSTEROID FRACTIONS (mg./24 hrs.)

	Pt.	Sex	Total	Non-ketonic	Alpha-ketonic	Beta-ketonic	% Alpha-ketonic	% Beta-ketonic
	Normal subjects		16.7	0.44	15.1	1.2	90.0	7.3
Laennec's cirrhosis	AL	M	9.8	0.62	6.7	2.5	73.0	23.7
	RM	M	5.5	0.18	4.0	1.3	68.0	25.7
	AS	M	5.1	0.75	4.4	0.0	85.0	
Undetermined cirrhosis	MS (after ACTH)	F	19.3	2.60	16.7	0.0	86.5	

The 17-ketosteroids are excreted for the most part in conjugated form. Approximately 50 per cent occur normally as glucuronidates, as indicated in our data and those of Buehler (4). Table 3 shows the "total" 17-ketosteroids after acid hydrolysis and the fraction released by bacterial glucuronidase in a similar specimen from the same collection of urine. In general, the absolute quantity and percentage of ketosteroids excreted as glucuronidates was much depressed in cirrhosis. Where two specimens of different

TABLE 3. 17-KETOSTEROIDS RELEASED BY BETA-GLUCURONIDASE

	Patient	Method of hydrolysis			
		Acid, mg.	Enzyme		None
			mg.	%	
	Normal subjects (2)			45–55	
Laennec's cirrhosis	ND	4.5	0.9	20.0	0
	MR	4.3	0.5	12.0	0
	MR	6.2	1.9	31.0	0
	AL	7.3	0.2	3.0	0
	AL (convalescent)	12.0	6.7	56.0	0
Undetermined cirrhosis	DM	3.9	0	0	0
	DM (1 day after ACTH)	9.5	2.1	22.0	0.1
	DM (2 days after ACTH)	12.8	3.5	27.0	0.1
	CG	4.8	0.9	19.0	0
	LB	1.6	0.2	12.5	0
Biliary cirrhosis	MH	3.5	0.3	8.5	0

total value from a single patient have been examined, the one of greater value has had a higher proportion of glucuronidate. This is also apparent after the increase in ketosteroids following ACTH. Almost none of the ketosteroids was detected as a free compound.

Lloyd and Williams (20) have reported a failure of 17-ketosteroids to increase following administration of testosterone to patients with Laennec's cirrhosis. This differs from the results of the present experience as indicated in Figure 2. Two of the patients studied in this regard had advanced cirrhosis; in fact, they succumbed a short time later.

Urinary corticoids

Corticoids (reducing) excreted per twenty-four hours prior to the institution of treatment were determined repeatedly in 7 patients with Laen-

nec's cirrhosis, 4 with cirrhosis of undetermined etiology, and 5 with biliary cirrhosis (Table 4). At this time, all patients were on a 1.0 Gm. sodium chloride diet with a fixed supplement of 8.0 Gm. of sodium chloride daily as enteric coated tablets. The corticoids appear to be elevated in all groups. L. B. is the single exception. This patient had been on a regular course of desoxycorticosterone and ascorbic acid for the treatment of concomitant arthritis shortly before these determinations. Occasional determinations of formaldehyde-generating steroids appear to confirm the presence of increased corticoids in the urine. The increase is in general less conspicuous

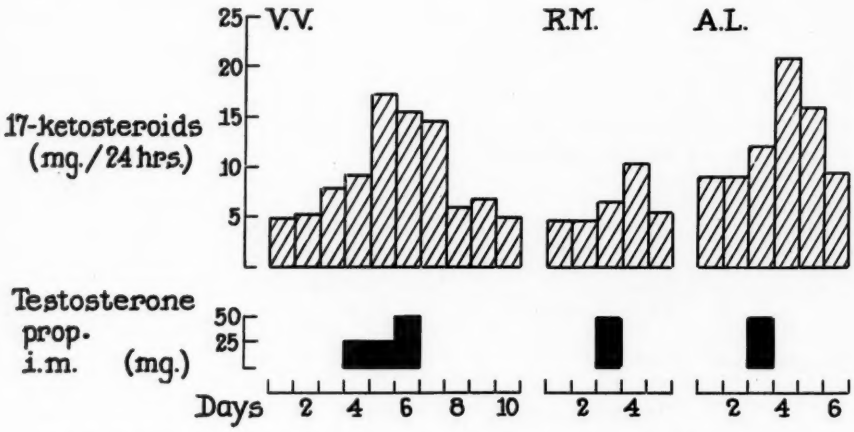

FIG. 2. Response of 17-ketosteroid excretion to the administration of testosterone propionate in 3 patients with Laennec's cirrhosis.

among patients with Laennec's cirrhosis. When repeated determinations have been performed, occasional peak levels are attained which well surpass the normal limits.

Bioassay of urinary corticoids

Physiologic identification of these steroids was sought in two ways. Hepatic glycogen bioassay in the mouse, according to the method of Venning (9), revealed values for urine extracts which were normal for the group with Laennec's cirrhosis but elevated in the second category (Table 4) comprised of young women with the interesting clinical features noted above. In addition to the method just mentioned, attempts to measure salt retention after the injection of these extracts into adrenalectomized rats (10) did not yield entirely satisfactory results because of a wide scattering of control values in spite of rigid technique. On this account, the

degree of retention of sodium, unless extreme, was not considered significant. Eight to 10 rats were used per assay. Thirty-minute aliquots of urine from 2 patients with Laennec's cirrhosis produced a significant degree of sodium retention in adrenalectomized rats. The effect appeared so marked as to suggest strongly the presence of "salt-water" active steroids in at

TABLE 4. DAILY URINARY CORTICOID EXCRETION IN CIRRHOSIS PRIOR TO DEFINITIVE THERAPY

	Pt.	Reducing corticoids (mg./24 hrs.)			Formaldehyde corticoids (mg./day) Avg.	Glycogen bioassay (gamma/14 hrs.)
		No. of analyses	Range	Avg.		
Adult normal subjects		8	1.0–2.0	1.38	1.4–2.0	437 (avg. of 6)
Laennec's cirrhosis	AS	3	1.6–2.2	1.90	2.6	318
	AL	4	1.2–2.3	1.83	1.2	
	VV	11	1.2–2.9	1.98		417
	WJ	1	1.7	1.70		480
	MR	4	1.0–3.2	1.65	2.3	110
	LS	1	1.9	1.90		
	ND	7	2.3–3.5	3.01	2.4	462
Undetermined cirrhosis	CG	8	2.0–3.7	3.02	4.2	3500
	MS	3	1.6–3.2	2.43		3000
	DM	8	1.6–6.3	3.49	3.4	1219
	LB	2	0.7–1.1	0.90		
Biliary cirrhosis	MH	1	2.0	2.00		800
	LG	2	3.3–5.0	4.65		
	MG	1	3.1	3.10		
	MP	3	2.5–3.4	2.97		
	SG	1	3.3	3.30		

least two of the urines. Other urines from this group with Laennec's cirrhosis similarly demonstrated some activity, but the variability of repeated controls did not allow sufficient precision to attach significance to the results.

Influence of rigid dietary restriction of sodium

In accordance with the procedure of Eisenmenger *et al.* (1), patients with various types of cirrhosis and ascites had their dietary sodium rigidly restricted after a preliminary period of stabilization. Five patients with

Laennec's cirrhosis were followed on this regimen with particular reference to their reducing corticoids (Fig. 3). A rise in the amount of these corticoids was noted following the institution of the low sodium intake, and was characterized by higher general levels and peaks than those observed prior

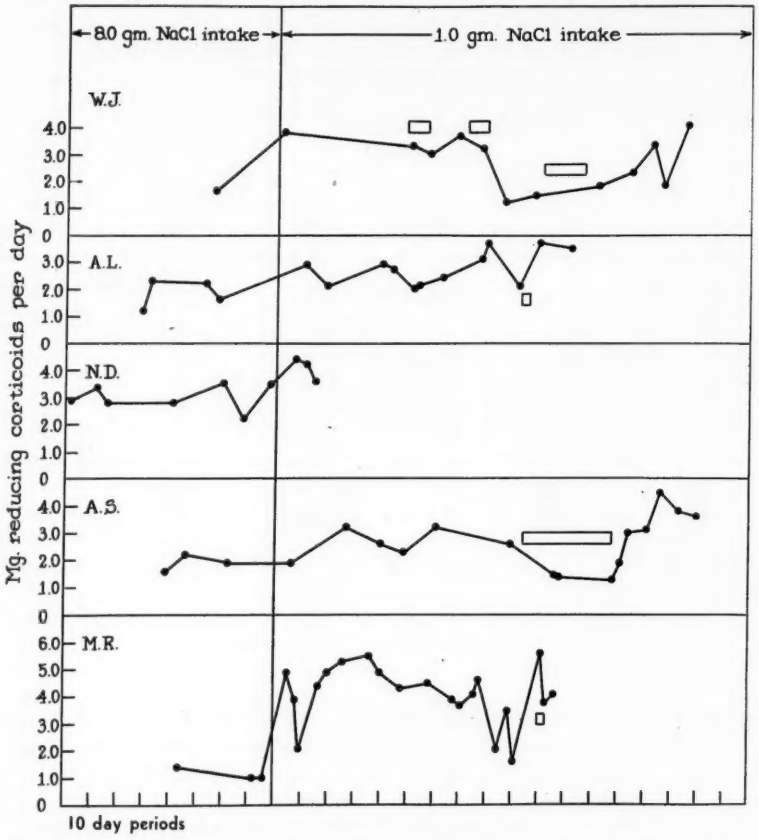

FIG. 3. The reducing corticoids in 5 patients with Laennec's cirrhosis before and after the institution of dietary sodium restriction. The blocks indicate intervals during which sodium was resumed (85 to 170 mEq. daily) as parenteral fluids or by mouth.

to this treatment except under conditions of stress. Although it is not constantly sustained, the general trend is one of increase. The total rise based on averaging all of the determinations (exclusive of those in which sodium was again added to the diet) in each individual patient was from 36 to 148 per cent. This rise after sodium restriction has been confirmed in one of these patients by Genest *et al.* (21).

Eosinophils

Eosinophil counts in this group of patients were generally depressed below the normal level. A moderate decrease following the intravenous administration of 0.6 cc. of adrenalin was demonstrated in four hours. Following ACTH this drop was more marked, falling to zero in each instance (Fig. 4).

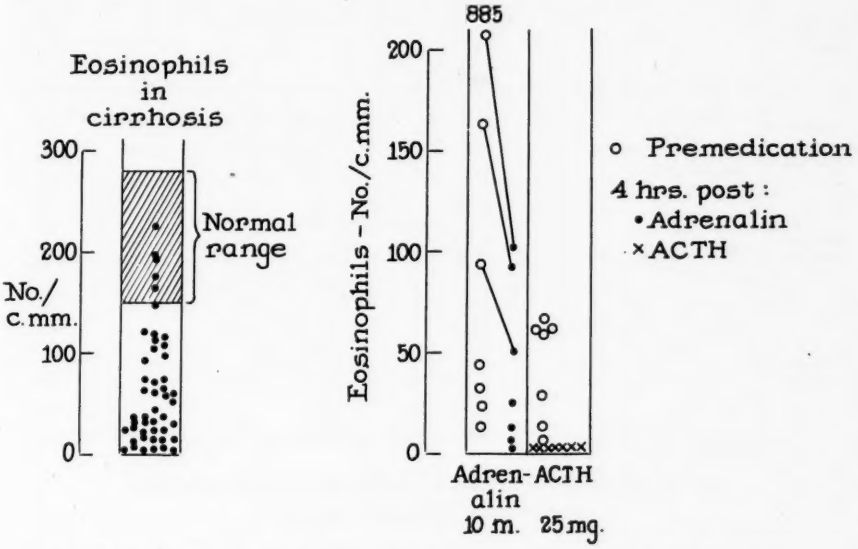

FIG. 4. Circulating eosinophils in patients with three types of cirrhosis and the response to adrenalin and ACTH.

Sodium metabolism

The phenomenon of salt and water retention in cirrhosis was apparent in the low urinary sodium (Table 6). That this salt retention is not exclusively renal, but part of a more generalized process, is evident from the low concentrations of sodium in saliva and sweat. This has been briefly summarized in Table 5 and is more fully presented elsewhere (22).

Effects of adrenocorticotropic hormone

The administration of adrenocorticotropic hormone to 7 patients with three types of cirrhosis resulted in changes noted in Tables 6 and 7. The hormone was given for short periods (six to twelve days) in doses of 100 mg. daily. In general, the usual effects of increased 17-ketosteroid and corticoid excretions, as well as rises in uric acid/creatinine ratios, were noted. No significant rise in creatine excretion was observed except in patient

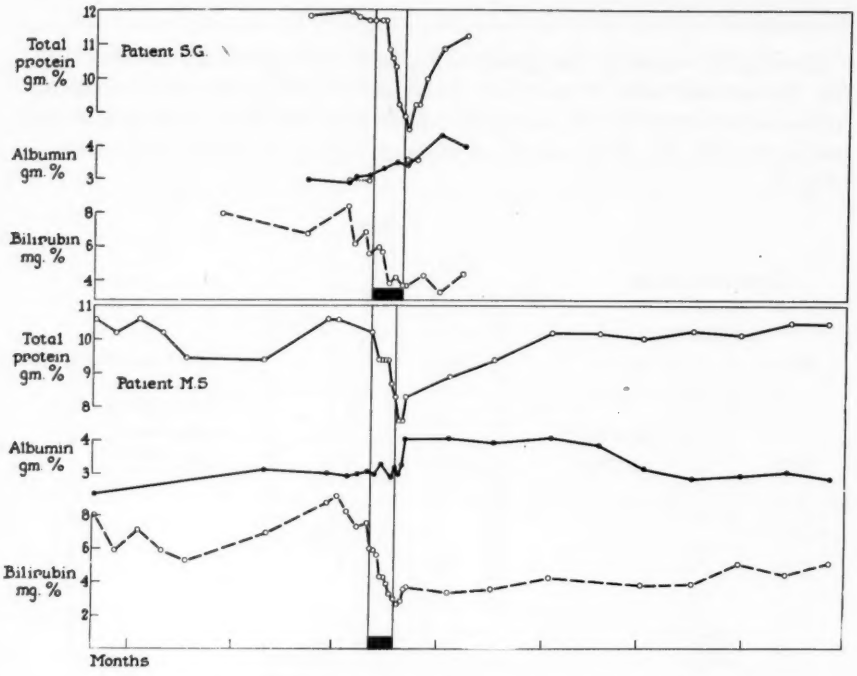

FIG. 5. The response to ACTH of 2 female patients with liver disease of undetermined etiology.

S. G., in whose urine a very marked elevation was found. The sodium concentrations in urine, saliva and sweat were diminished except in V. V., whose urine showed a previous markedly depressed sodium value. It should be noted that the sweat figures on V. V., although initially low in sodium, are not included since it was not possible to induce perspiration

TABLE 5. SODIUM CONCENTRATION IN SALIVA AND SWEAT

	Saliva			Sweat		
	No. of patients	Sodium (mEq./L.)		No. of patients	Sodium (mEq./L.)	
		Range	Avg.		Range	Avg.
Normal subjects	10	13.1–41.0	22.6	4	12–95	60
Laennec's cirrhosis	6	2.7–31.0	13.1	3	4–49	16.9

TABLE 6. OBSERVATIONS ON ACTION OF ACTH IN PATIENTS WITH CIRRHOSIS

	Pt.	*Pre-ACTH* (3–5 day intervals) Average	Range	*During ACTH* (1 day intervals)									*Post-ACTH* (4–5 day intervals)						
17-Ketosteroid excretion mg./24 hrs. (*see* Table 1)	VV*	5.3	(3.6–6.9)	8.9	14.6	14.8	14.7	17.2	17.5				8.2	8.6	4.7	7.8			
	MS†	8.6	(7.0–11.5)	10.3	17.1	19.3	24.3	18.3	30.0				11.9	6.8					
	SG‡	3.7	(3.7)	6.6	11.4	22.3	19.1	16.3	30.2	20.0				8.5	2.4	3.6			
	LB†	4.0	(4.0)	10.7		8.2		12.9					1.6						
	DM†	5.2	(3.9–7.0)	9.5		12.8	18.0	14.8					14.0	5.0	7.0				
Reducing corticoids mg./24 hrs. (*see* Table 4)	VV*	2.0	(1.5–2.6)		3.8		4.0		2.9				2.6	2.6	1.8				
	MS†	2.1	(1.6–2.5)		4.5		5.8						3.2		2.5				
	SG‡	3.3	(3.3)		3.5		8.2							1.4					
	LB†	0.9	(0.7–1.1)		3.1		3.8							0.9					
	MH‡	2.0	(2.0)		3.5		4.4							1.8					
	DM†	2.3	(1.6–2.7)		4.5			10.5						4.0	6.3	4.4	3.3		
Urinary uric acid creatinine ratio	VV*	0.36	(0.24–0.29)	0.45	0.44	0.44	0.42	0.46					0.40	0.33					
	MS†	0.41	(0.37–0.43)	0.48	0.47	0.53	0.53	0.53	0.99				0.45						
	SG‡	0.32	(0.26–0.36)		0.84	0.96	1.79	1.27					0.31	0.35					
	DM†	0.25	(0.24–0.25)	0.57	0.41	0.44								0.39	0.40	0.28			
Creatine mg./24 hrs.	VV*	883	(814–952)			929	935							819					
	MS†	206	(177–235)			108	327												
	SG‡	324	(259–363)		844	962	1790		1272				1185	312					
	DM†	601	(489–714)	739	452		625	841						449					
Urine sodium mEq./24 hrs.	VV*	0.13	(0.11–0.17)	0.15	0.12	0.14	0.18	0.26	0.25				0.33	0.53	0.71	0.29	0.21	0.66	0.19
	MS†	71.9	(69.2–73.6)	95.7	51.0	28.5	33.1	51.8	51.3	54.2				62.2	369.0	292.0	284.0	33.6	
	LB†	105.1	(91.3–119.0)	72.5	16.3	10.4	9.1	2.9	1.3	1.2	0.76	1.4				6.9			
	DM†	1.54	(1.07–1.82)	0.55	0.55	0.33	0.22	0.18	0.45				1.79	2.04	1.75	1.71	1.75		
	MH‡	113.4	(23.3–176.0)	8.8	2.6	3.7		7.7	2.1				156.0	58.5	124.0	144.0	168.0	130.0	
	FW†						17.6	40.7	54.5				16.2	10.8	23.0				
Serum sodium and potassium mEq./L.	VV*	131.1	(131.0–131.2)		128.4	130.0	128.4	128.5	133.4					133.4	134.5				
		4.7	(4.5–4.8)		4.5	4.5	4.6	4.8	5.1					4.2	4.5				
	MS†	134.6	(134.2–135.0)	133.3	131.0	133.8		137.5	137.5					135.3					
		3.5	(3.5)	4.0	3.7	3.5	3.6	3.8	3.3					3.7					
	SG‡	128.0	(128.0)	125.2		127.5		130.5	131.6						124.2				
		4.3	(4.3)	4.0		3.8		3.4											
	MH‡	138.5	(135.4–141.6)	137.6	138.7	136.5	139.0	136.0					134.5	135.6		140.0			
Serum bilirubin mg./100 cc.	VV*	1.0	(0.9–1.3)		1.2		0.8	0.6	0.8				0.8	0.7		1.4	1.3	1.3	
	MS†	7.8	(6.5–9.2)	6.0	5.9	5.6	4.3	4.3	3.9				3.3	2.7	2.9	3.6	3.4	3.6	4.3
	SG‡	6.9	(5.6–8.4)		5.7		4.2		3.8				3.7		4.3		3.3		
	LB†	1.6	(1.6)	1.2			1.3	1.5	1.4						1.2				
	MH‡	9.6	(9.4–9.7)		9.1	9.4	8.9		9.1					15.0		16.0	13.2		
	FW†	12.6	(11.6–13.6)	13.6		11.1								8.4	8.9				
	DM†	2.3	(1.2–2.4)	1.2		1.0	1.2		0.9					1.2					
Serum total protein and albumin Gm./100 cc.	VV*	5.73	(5.73)			6.1								5.38	6.1	5.73		5.38	
		1.8	(1.8)			2.15								2.05	2.0	2.3	2.1	1.8	10.0
	MS†	10.1	(9.4–10.6)	10.4		9.4		8.2					7.6	7.6	8.8	9.2			3.8
		2.9	(2.5–3.0)	2.9		3.3		3.35					3.2	4.0	4.0	3.9			
	SG‡	11.9	(11.7–12.0)			10.8		9.25						8.5	8.15		10.0		
		1.7	(1.5–1.75)			2.08		2.35						2.5	2.85		3.36		
	LB†	6.7	(6.3–7.0)	6.85		7.6	7.2		7.0				6.85		7.2			5.85	
		3.4	(3.2–3.51)	3.33		3.65	3.61	3.63	3.7				3.88		4.13	4.18		4.3	
	DM†	7.6	(7.6)		7.6									7.2		6.85	6.85		
		2.4	(2.3–2.4)		2.5			2.29						2.95	2.54	2.7	2.84		
	MH‡	7.1	(6.85–7.2)			7.0	6.85	5.4						5.73					
		2.6	(2.5–2.6)	2.75		2.65	2.81	2.9						2.87					
Saliva sodium and potassium mEq./L.	VV*	9.0	(9.3–12.1)		2.4	2.3	2.4	2.3					6.8	4.2	14.6		4.2		
		33.4	(25.0–43.0)		27.0	32.0	29.0	35.0					39.0	34.0	33.0		27.0		
	DM†	11.1	(9.6–14.0)	7.8		2.8		6.8					5.4	13.6	16.8	18.4	14.0		
		16.2	(15.0–18.0)	17.2		20.0		22.2					22.2	20.4	21.8	19.6	18.2		
	MH‡	30.6	(30.6)		11.6		14.4							19.6	13.4	21.4	16.0		
		21.4	(21.4)		27.2		26.2							22.0	21.0	29.8	24.4		
Sweat sodium and potassium mEq./L.	MS†	52.0					6.5								24.0				
		33.0					4.5								8.0				
	SG‡	23.5	(21.0–26.0)		16.5		21.0												
		12.5	(5.0–20.0)		6.2		6.0												

* Laennec's cirrhosis.
† Undetermined cirrhosis.
‡ Biliary cirrhosis.

during the administration of ACTH. Patients with Laennec's cirrhosis and fluid retention in general perspire poorly and improvement in this condition was frequently attended by an increase in this function. The saliva potassium showed a tendency to increase during administration of ACTH. Patients M. S., S. G., and F. W., with cirrhosis of undetermined etiology and initially high serum bilirubin levels, demonstrated a decrease of this substance in the serum during therapy, a decrease which continued for a fairly prolonged period after cessation. The rise in serum albumin in patients M. S., S. G., L. B. and D. M. was notable and appeared to continue well after cessation of ACTH.

TABLE 7. EFFECTS OF ACTH ON SERUM LIPIDS OF PATIENT WITH LOW INITIAL VALUES AND CIRRHOSIS OF UNDETERMINED ETIOLOGY

Patient MS	Date	Total cholesterol	Total phospholipid	Total
		mg. %	mg. %	mg. %
Before treatment	12/ 9/49	112	181	487
ACTH (100 mg. daily)	12/19/49	134	213	608
	12/22/49	150	290	690
After treatment	1/ 4/50	171	345	800
	1/17/50	156	280	695

Thus, in some patients certain evidence of improvement was found during ACTH treatment as indicated by a drop in serum bilirubin and a rise in serum albumin, the latter having been demonstrated by four different methods. It is of interest that these signs of improvement occurred only in the group of patients with cirrhosis of undetermined etiology. A fall in total protein and gamma globulin occurred concomitantly. These beneficial reactions were accompanied by general clinical improvement. Plasma volumes, determined by means of the dye T1824, rose as much as 10 to 20 per cent, which further emphasizes the rise in serum albumin. The changes persisted well after therapy was discontinued, although the plasma volume returned to normal. However, the alterations were not permanent and several weeks later the protein pattern reverted to its initial picture. Figure 5 illustrates the phenomena in 2 representative patients and Figure 7 reveals the electrophoretic patterns in M. S. before and after administration of ACTH. The results in D. M., V. V. and M. H. were somewhat obscured by the sudden onset of acute abdominal pain accompanied by the appear-

ance of ascites, which was bloody in 2 of the patients. This necessitated discontinuation of therapy and attention to the complications.

The complications attending ACTH administration in cirrhosis were occasionally severe and constituted a major limitation in the conduct of this study. Edema, salt and water retention, increased plasma volumes and

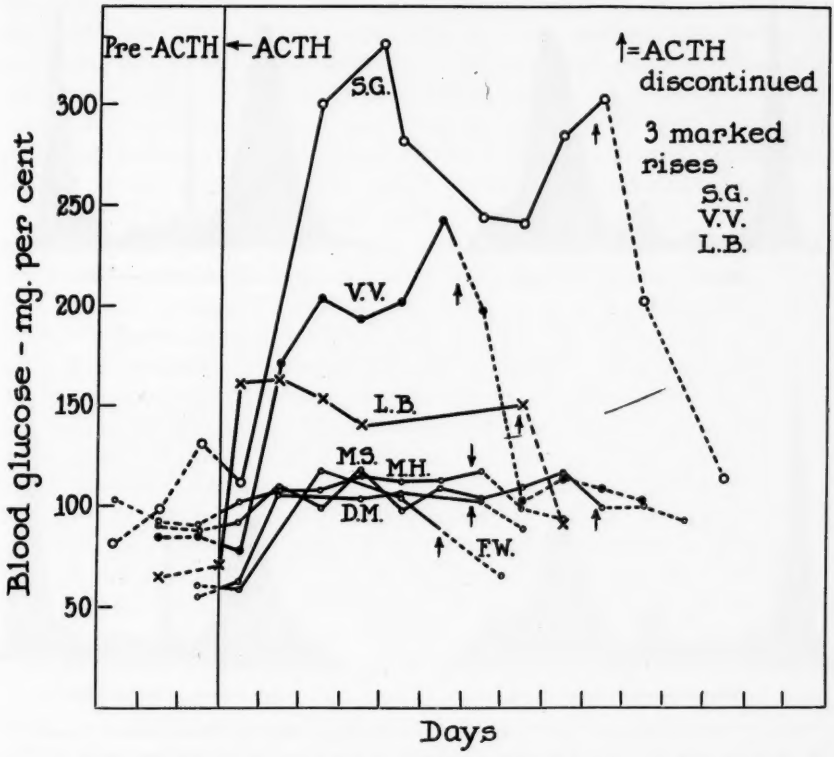

FIG. 6. Fasting blood glucose levels in 7 patients with three types of cirrhosis on short-term ACTH therapy. In 3, the rise is well beyond normal limits.

acneiform eruptions such as have been repeatedly described, were seen in this group. These alone were of no serious consequence except for the failure of some acneiform lesions to localize. However, of major importance has been the sudden onset of severe abdominal pain in 3 patients during ACTH therapy, accompanied by bloody ascites in 2. In one (M. H.) with biliary cirrhosis, ascites had never been present previously but developed suddenly during therapy. In another (V. V.) with severe pre-existing as-

cites, there was hemorrhage into the abdominal cavity; this patient succumbed three weeks later, following a respiratory infection and hepatic coma. At autopsy there was thrombosis of the portal vein, of recent origin, extending into some of the mesenteric radicals. Its formation seemed to have occurred concurrently with the administration of ACTH. In the third

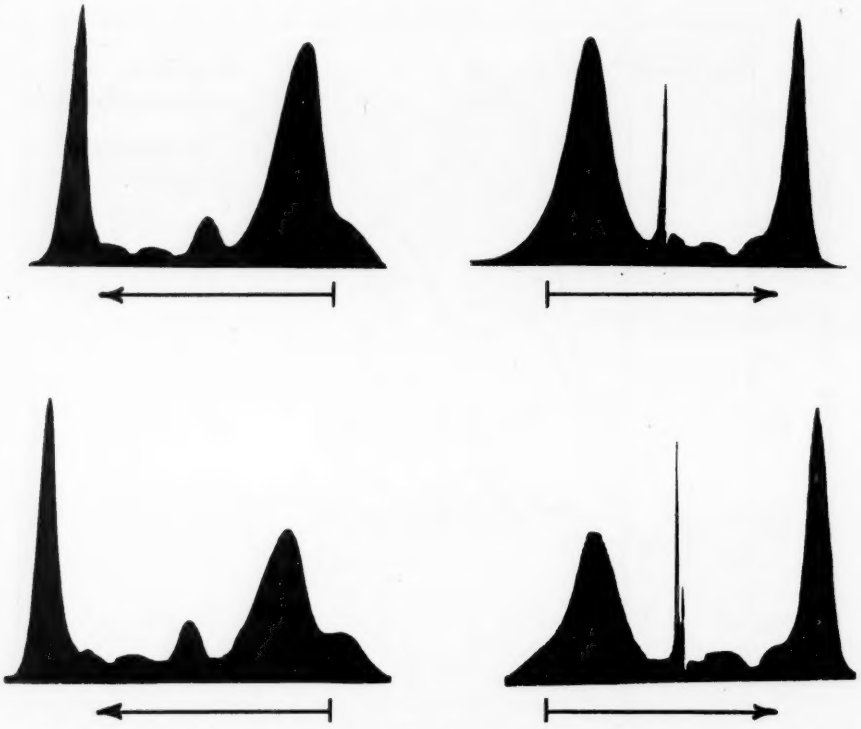

FIG. 7. Ascending and descending electrophoretic patterns on the serum of patient M. S. with cirrhosis of undetermined etiology before and during therapy with ACTH. The liver patterns show the change in the ratio of the albumin and gamma globulin after six days of therapy. These changes resulted from a 15 per cent rise in plasma volume, a rise in albumin and a fall in gamma globulin.

patient (D. M.) heparin therapy was begun immediately upon the onset of abdominal pain and moderate ascites. Within two weeks improvement occurred.

The response of fasting blood glucose levels to ACTH is seen in Figure 6, wherein the abnormally high levels attained by V. V., S. G., and L. B. are prominent. It is stressed that these phenomena occurred on ordinary doses of ACTH within a short time after initiation of treatment. These levels

are unusually high and their appearance in 3 of 7 patients treated seems significant. In only 1 was there a suggestion of diabetes in the family history, but this was questionable.

DISCUSSION

The low excretion of 17-ketosteroids noted above, particularly in those patients with Laennec's cirrhosis, is in keeping with the findings of Lloyd and Williams (20). Bioassay according to these workers has shown, in addition, low levels of androgenic activity in urines from patients with Laennec's cirrhosis. This suggests a diminished output of ketosteroids by the adrenals in all patients of this group, and possibly also by the testes in the male. Forbes *et al.* (23) have called attention to low urinary 17-ketosteroids in chronic illness generally. Thus, the state of debilitation present in the patients of the present study upon admission may account for the low excretion of urinary 17-ketosteroids. A. L., the single convalescent patient, showed a normal level of ketosteroids. Such depression of ketosteroid excretion alone is not an indication of general adrenal hypoactivity, since it reflects only one aspect of adrenal function. In fact, it is conceivable that adrenal secretion of ketosteroids is not diminished, but that proper disposal as water-soluble conjugates in the urine is compromised. The role of the liver in glucuronide synthesis is well established (24). The small amount of ketosteroids excreted as glucuronidates in liver disease (Table 3) suggests failure of the diseased liver to conjugate these compounds as glucuronidates, resulting possibly in the formation of other end products not cleared by the kidneys. A higher total ketosteroid excretion is accompanied by a higher percentage of glucuronidate in A. L. during convalescence and in M. S. after administration of ACTH. In spite of the low basic excretion of 17-ketosteroids in cirrhosis, in our experience a rise has followed the administration of testosterone propionate. The response of the adrenal to ACTH in the three types of cirrhosis, as manifested by rises in various steroids and disappearance of eosinophils, certainly rules out primary adrenal failure as an explanation of the basically low ketosteroid excretion in this disease. The wide, daily scattering of 17-ketosteroid secretion in these patients, which seems related to urine volume, has been noted in normal individuals by Guinet *et al.* (25) and by Neukomm and Reymond (26). These French workers remark on the very close direct relationship between urine volume and ketosteroid excretion. This has been little noted by others. Patients with cirrhosis having disturbances in salt and water metabolism exhibit occasionally great variations in urine volume; usually small volumes with intermittent bursts of diuresis. Perhaps the variations in 17-ketosteroids with urine volume are not peculiar to the disease but are expressions of a normal phenomenon. The rise of urea excretion with di-

uresis is well known and it is possible that the ketosteroid variation is on a similar basis. The lowest excretion of 17-ketosteroids has occurred in those patients with cirrhosis and fluid retention, and may represent a consequence of the low urine volume.

The elevation of "corticoids" in the three types of cirrhosis, determined chemically, is of interest. Although the method of Heard and Sobel was most often used, confirmation was secured by the measurement of formaldehyde-producing steroids in several specimens. It cannot be stated at present whether these findings reflect an increased production of corticoids by the adrenals or a failure of their proper metabolism. In Laennec's cirrhosis with fluid retention, it is believed that these substances may be identified with "salt and water" hormones on the basis of a high degree of physiologic activity of this type in at least 2 patients. Urines of the other patients with Laennec's cirrhosis have also shown such an effect but because of the difficulty with controls, their precise importance cannot be determined. Deming and Luetscher (10), in their preliminary studies, report the presence of such activity in edematous states. The sodium retention in cirrhosis with ascites could then be, at least in part, an expression of increased adrenal desoxycorticosterone-like hormones. The low sodium concentration in sweat and saliva which we have demonstrated points to a general mechanism of sodium retention in this disease, not simply a renal one. This could best be explained by an hormonal influence and, in fact, low sweat sodiums have been regarded by Conn (27) as an index of increased "salt and water" hormonal activity of the adrenal cortex. Furthermore, the rise in urinary reducing corticoids during sodium restriction in these patients suggests an increased cortical production of "salt and water" hormones in response to the need for further sodium conservation. This combination of data strongly implicates the adrenal cortical hormones in the alterations of salt and fluid control in Laennec's cirrhosis with ascites.

The rise in reducing corticoids during sodium restriction in these patients has been supported by studies of Genest *et al.* (21) on a single patient in this group. Using a chromatographic column for the separation of urinary steroids, they have found a rise in a well defined, but as yet unidentified, group of compounds following salt restriction. The exact significance of this is yet to be elucidated, as well as its possible role in the eventual improvement of certain patients following prolonged sodium restriction in cirrhosis (Eisenmenger *et al.* (1)). Daughaday and MacBryde (28) have failed to detect, with one exception, a change in formaldehydogenic steroids following sodium restriction. This exception was in a female with manifestations of Cushing's syndrome and initially elevated urinary corticoids; she resembled our patients with cirrhosis in this respect. It may be that such rises on restricted sodium intake occur only when the original

levels are high. In addition, they reported a cyclic variation in their one patient so that the rise was not constantly sustained. This is also comparable to our experience. It is difficult to draw more minute comparisons with their observations because of differences in methods and a considerably longer period of restriction of sodium in our group. It is possible that the results from reducing methods for corticoids are more susceptible to variation after restriction of sodium than are those depending on the release of formaldehyde by periodic oxidation. The initially low eosinophil counts in cirrhosis also suggest adrenal cortical hyperactivity, but not of the type specific for desoxycorticosterone.

Patients with cirrhosis of the three types studied are capable of responding to adrenocorticotropic hormone with a rise in excretion of steroids, increased uric acid/creatinine ratio, and a fall in eosinophils. It is concluded that the adrenals are responsive to this direct stimulus even in patients with severe hepatic impairment. The rise in urinary steroids in cirrhosis after administration of ACTH would seem to contradict Conn's (29) prediction that such could not occur in hepatic disease owing to a deficiency of cholesterol esters in serum which, according to him, represent precursors of adrenal steroids.

Attention is called to the marked rise in blood glucose immediately after the initiation of ACTH therapy in 3 of 7 patients treated by us. These patients belonged, one each, to the three types of cirrhosis studied. Conn (30) has reported the production of temporary diabetes mellitus with ACTH in normal volunteers as determined by the finding of abnormal glucose tolerance curves. The fasting blood glucose levels rose little, if at all, above 120 mg. per cent. If this rise is due to gluconeogenesis, then the processes involved in glucose production are intact in cirrhosis. Thorn (31) believes that the hyperglycemia during glucose tolerance studies after administration of ACTH is related to factors other than decreased utilization of carbohydrate, because there is a fall in the amount of serum inorganic phosphorus during tolerance tests early in the course of administration of the drug. It is thought that the significant rises in fasting serum glucose of our 3 patients may be related to the inability of the diseased liver to accommodate large quantities of glycogen.

It must be stressed that the hazards of ACTH therapy in cirrhosis appear to be great. In our experience there seemed to be a tendency for portal thrombosis to take place; this occurred in 3 patients. In a fourth patient, severe fatal esophageal hemorrhage developed on doses of ACTH less than 50 mg. daily. This may have had no relation to ACTH therapy, although the patient had never bled previously. The general use of ACTH in chronic liver disease, on the basis of these few cases, seems to be contraindicated.

From observations with short-term therapy, it appears that ACTH does

not benefit patients with Laennec's cirrhosis. Except for a temporary effect on blood volume, which rose, there was little alteration in the status of the excessive extracellular fluid compartment. Substantial clinical and laboratory improvement, although temporary, was seen only in the group of young women with unusual liver disease characterized by extreme aberrations in serum proteins (32).

Glycogen deposition in adrenalectomized mice as a means of assessing action of cortisone-like elements in the urinary corticoids has shown normal amounts in Laennec's cirrhosis but significantly elevated levels in the group of young females with disease of undetermined etiology. The latter group has shown moderate, if any, retention of sodium in urine, sweat and saliva, and there has been no evidence that their urine extracts produce a diminution of sodium excretion in adrenalectomized animals as was found to be the case in the urine extracts from 2 patients with Laennec's cirrhosis. The elevated corticoids in this group are probably of a different physiologic type and, if comparable to compound "E," explain some of the Cushing's disease-like features seen in these patients.

CONCLUSIONS

The depressed daily excretion of 17-ketosteroids and elevated "corticoids" in chronic hepatic disease indicate that there are disturbances in the metabolism of adrenal cortical hormones.

The diminution in the proportion of ketosteroids excreted as glucuronidates in hepatic cirrhosis of all types implies interference with their disposal as water soluble metabolites. The low alpha-ketosteroids are primarily responsible for the decreased total quantity. Testosterone propionate has provoked a moderate rise in the excretion of 17-ketosteroids in cirrhosis. Ketosteroid excretion varied directly with urine volume.

The concentrations of sodium in urine, saliva and sweat are markedly reduced in cirrhosis with fluid retention. Some evidence has been obtained that the elevated urinary corticoids in Laennec's cirrhosis are physiologically related to desoxycorticosterone-like hormones of the adrenal cortex. A further rise in chemically determined corticoids was observed after sodium restriction.

Attention is called to a group of young females with chronic hepatic disease of unknown etiology, having clinical features of hyperadrenalism early in the course of their disease. Most characteristic is the elevation of serum gamma globulin and total protein. Urinary corticoids are high in these patients and biologic tests indicate increased glycogen storage activity. The exact relationships in this unique syndrome remain to be clarified.

Adrenocorticotropic hormone produced the expected metabolic changes in patients with chronic hepatic disease of the three types studied. In addi-

tion, marked elevation of fasting blood glucose has been observed in 3 of 7 patients and may be related to failure of hepatic glycogen storage. No significant benefit was seen in patients with Laennec's cirrhosis. The complications of ACTH therapy in patients with hepatic cirrhosis are serious and constitute a contraindication to its regular use in this condition.

Temporary beneficial effects consisting of a fall in serum bilirubin, a rise in serum albumin, and a fall in serum gamma globulin were observed in the group of young females with cirrhosis of unknown etiology who received ACTH therapy.

Acknowledgment

The authors are indebted to Doctors H. G. Kunkel and S. H. Blondheim for invaluable assistance in this study.

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THE EFFECT OF TESTOSTERONE ON THE PITUITARY-ADRENAL CORTEX MECHANISM IN NONCANCEROUS AND IN BREAST CANCER SUBJECTS*

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FORSHAM *et al.* (1) have demonstrated that the administration of 11, 17-oxysteroid hormones of the adrenal cortex causes a fall in circulating eosinophils and that this effect can be produced by adrenal stimulation, using adrenocorticotropic hormone (ACTH). Subsequently Recant and her coworkers (2, 3) reported that the administration of epinephrine stimulated production of ACTH by the pituitary and thereby, in the presence of an intact pituitary-adrenal cortex mechanism, produced a fall in eosinophils. This reduction approximates 50 per cent or greater in four hours following the administration of epinephrine in subjects with normal pituitary-adrenal relationships. Similar reductions in circulating eosinophils have been obtained by Laragh and Almy, using insulin intravenously (4).

The use of epinephrine and insulin in studying pituitary and adrenal function has been suggested by Thorn (5), and Laragh and Almy (4). Therefore an investigation of the response of the pituitary-adrenal system to epinephrine stimulation in patients receiving testosterone propionate has been undertaken. This response has been measured by determining the effect of epinephrine in producing a fall in eosinophils before, during and after the administration of testosterone to human subjects. In view of the current interest in the use of both androgens and estrogens in the palliation of advanced breast cancer we have attempted to correlate our results with the clinical findings in 6 patients with this disease.

Previous work by Venning and Browne (6) has indicated that testosterone reduced the urinary excretion of 11-oxysteroids. This has been con-

Received for publication July 15, 1950.

* This investigation was aided, in part, by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

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firmed by Talbot *et al.* (7). The latter have postulated that the administration of testosterone produces inhibition of the pituitary and decreases the ACTH production of that gland, thereby reducing the production of glycogenic steroids by the adrenal. Also, Sayers and Sayers (8), using the method of measurement of adrenal ascorbic acid and cholesterol depletion under stress of various types, have found that pituitary ACTH production is inhibited following the administration of such compounds as 17-hydroxycorticosterone, 17-hydroxy-11-dehydrocorticosterone, corticosterone and desoxycorticosterone. Their data suggest that there is a direct action of these steroids in decreasing pituitary ACTH production. This has been found by Thorn *et al.* to be true also in humans receiving 17-hydroxy-11-dehydrocorticosterone (9). Bartter *et al.*, and Lewis and Wilkins, working with patients suffering from Cushing's syndrome, have found that testosterone inhibits the classical ACTH effects found in Cushing's disease but when parenteral adrenocorticotropic hormone (ACTH) is given simultaneously, there is no inhibition of the ACTH effect (10, 11). These investigations suggest that testosterone reduces the ability of the human pituitary to elaborate ACTH. In addition, there is evidence from human and animal studies that testosterone diminishes other pituitary functions and that not only ACTH but LH and FSH production is inhibited (12–15).

METHODS

Ten female patients ranging from 44 to 70 years of age were studied during the administration of testosterone propionate[1] in oil in doses of from 12.5 to 100 mg. intramuscularly daily. Those included were 6 with carcinoma of the breast, 1 with carcinoma of the cervix, 2 with fractures of the femur (convalescent), and 1 with leg ulcers secondary to varicose veins. One patient with a fracture of the femur and 1 with quiescent pulmonary tuberculosis were studied as controls without receiving testosterone during repeated epinephrine tests. Epinephrine tests were made at two to ten-day intervals and in 7 patients were performed before the administration of testosterone propionate in order to establish control levels of eosinophil destruction. Epinephrine hydrochloride (1:1000 adrenalin) was administered subcutaneously in 0.3 cc. (0.3 mg.) doses in all tests and blood was drawn before and four hours after the administration of the drug. The direct counts of circulating eosinophils were performed by Dun-

[1] This investigation was done under the auspices of the Therapeutic Trials Committee of the Council on Pharmacy and Chemistry of the American Medical Association as part of its collaborative study of steroids and cancer.

We are indebted to Dr. Edward Henderson of Schering Corporation, Bloomfield, N. J., for supplies of Oreton and to Dr. Kenneth W. Thompson of Organon, Inc., Orange, N. J., for Neo-hombreol.

ger's method modified by Hills, Forsham and Finch (16). Adrenocorticotropic hormone[2] was given in 25-mg. doses intramuscularly and insulin was administered intravenously to patients in the fasting state in doses of 0.1 unit per kilogram of body weight.

RESULTS

The results of the repeated epinephrine tests are summarized in Table 1. Of 10 patients studied, repeatedly subnormal responses to epinephrine while receiving testosterone were found in 6. These patients demonstrated a fall in eosinophils which was less than the normal 50 per cent after the

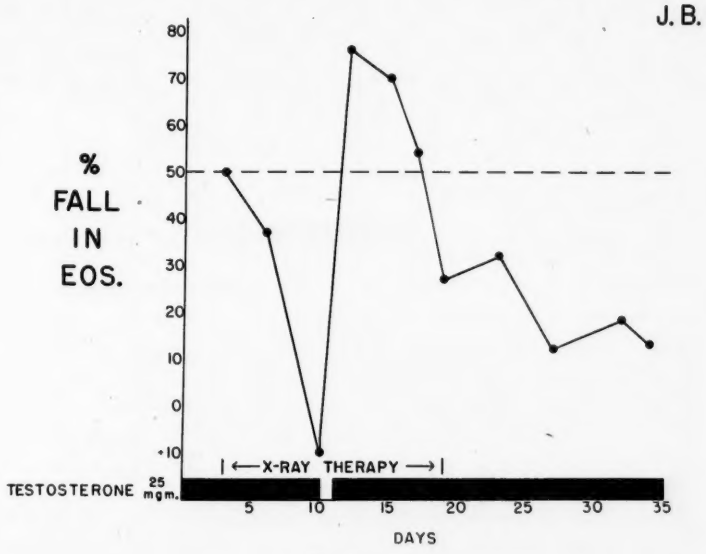

FIGURE 1

administration of epinephrine. Four patients studied over periods of nine, fourteen, eighteen, and forty-nine days consistently showed a fall of 50 per cent or greater in eosinophils in spite of the administration of testosterone. The subnormal response which was demonstrated in 6 patients suggests that the administration of testosterone produced diminished activity of the pituitary-adrenal system.

The diminished fall in eosinophils was first observed in patient J. B. (Fig. 1) in whom subnormal epinephrine responses occurred early in tes-

[2] Lot No. H-3907 obtained through the courtesy of Dr. John R. Mote, Armour Laboratories, Chicago 9, Ill.

In the article entitled "The Effect of Testosterone on the Pituitary-Adrenal Cortex Mechanism in Noncancerous and in Breast Cancer Subjects" by W. W. Faloon, L. A. Owens, M. C. Broughton and L. W. Gorham, in the same issue, page 173, the second and third footnotes in Table 1 should be reversed.

TABLE 1

		Breast carcinoma												Ca. cervix		Fractured femur		Fractured femur		Leg, ulcer	
Patient →		K.B.		I.G.		E.L.		R.M.		E.M.		F.P.		J.B.		R.W.		H.G.		F.S.	
Daily dose →		50 mg.		50 mg.		50 mg.		50 mg.		12.5–25 mg.		50–100 mg.		25 mg.		50 mg.		50 mg.		50 mg.	
Eosinophils →		Init. cnt.	% Fall	Init. cnt.	% Fall	Init. cnt.	% Fall	Init. cnt.	% Fall	Init. cnt.	% Fall	Init. cnt.	% Fall	Init. cnt.	% Fall	Init. cnt.	% Fall	Init. cnt.	% Fall	Init. cnt.	% Fall
Days before testosterone	5					125	53			156	76										
	4					94	60			128	74									383	53
	3			168	52																
	2															522	63				
	1	207	55															203	80	275	46 (late)*
Days of testosterone	1											50 mg.				443	88				
	2	241	60									50 mg.									
	3			187	61	121	46			100	56	50 mg.		212	49					224	29
	4											50 mg.						162	53		
	5	183	57	82	45							50 mg.									
	6					150	50			120	66	100 mg.		104	37						
	7	170	69									100 mg.†						112	37	261	40
	8															425	43				
	9	226	45					150	43			137	36					81	60	287	36
	10			118	60	131	60			108	84			76	+10	268	2				ACTH
	11	135	26											Testost.‡ omitted						150	59
	12											137	13	232	76					220	84 Insulin
	13	212	50					168	31												
	14			143	66	124	53			47	77									225	14
	15	240	32											179	70						
	—																				
	17											150	33	175	54						
	18	230	ACTH 87			146	71			91	57										
	19																			228	46
	20	126	36											175	27					Testos. stopped	
	21	206	56 Insulin			135	53			78	38										
	22																			223	42
	23													367	32						
	24	128	65																		
	25					152	63														
	27													148	11						
	28					139	60														
	30																			240	29
	31					119	60														
	32													150	16						
	34					138	66							337	13						
	36																			361	58
	38					148	34														
	40					145	54														
	44																			284	66
	45					136	62														
	49					212	16														

* Second specimen drawn 40 minutes late.
† Testosterone omitted for one day.
‡ 100 mg. daily after this date.

tosterone therapy. On the twelfth, fifteenth and seventeenth days, however, after the omission of testosterone for one day, normal eosinophil falls were recorded. This was followed by a period of inhibited response to epinephrine which continued through the thirty-fourth day, when studies were stopped. Examination of the absolute eosinophil counts before epinephrine stimulation, however, reveals that relatively low numbers of eosinophils were present during the sixth to tenth days. This may indicate a period of stress during this period which precluded further stimulation by epinephrine. The possible mechanism of the irregularity found in this patient is discussed below.

In order to demonstrate the site of the block to epinephrine which occurred when testosterone was given, adrenocorticotropic hormone was administered parenterally to 2 patients. After these had repeatedly demon-

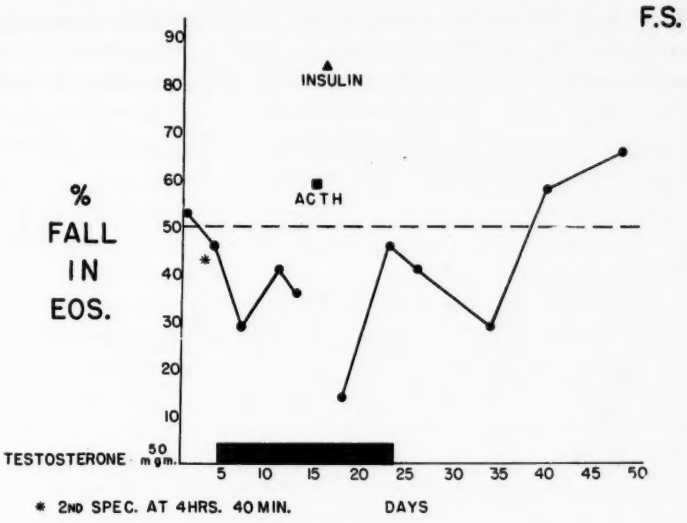

FIGURE 2

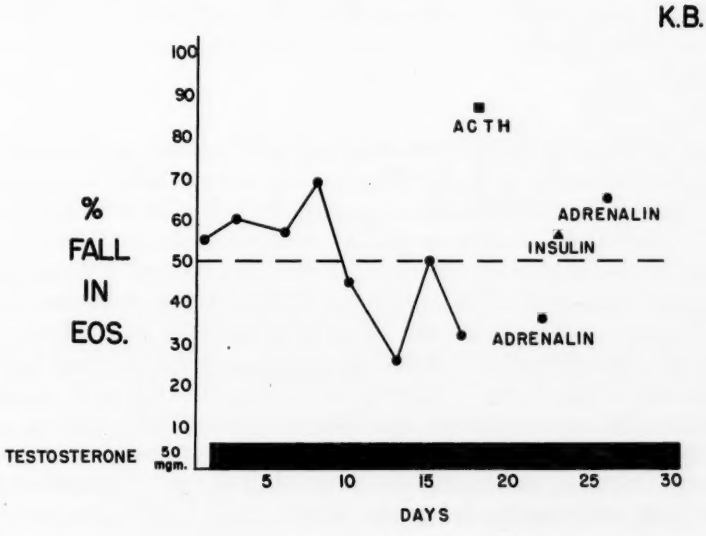

FIGURE 3

strated eosinophil falls of less than 50 per cent following epinephrine, ACTH was administered intramuscularly in 25-mg. doses. At this time, the injection of ACTH produced a normal fall in eosinophils (Figs. 2 and 3). Thus it appears that the administration of testosterone does not inhibit the action of ACTH on the adrenal cortex but instead decreases the production of pituitary ACTH following epinephrine stimulation. In order to determine whether the block of epinephrine action on the pituitary was complete and active for other types of stress-producing substances, insulin was given intravenously to the same patients (Figs. 2 and 3). Normal falls in eosinophils were produced by this measure.

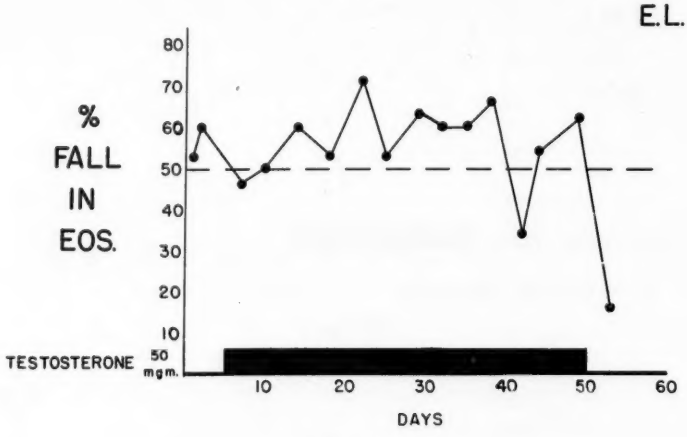

FIGURE 4

That the effect of testosterone on the pituitary is not permanent is also demonstrated in patient F. S. (Fig. 2). This subject failed to respond to epinephrine with a normal drop in eosinophils during the administration of testosterone but did respond to ACTH and to insulin. After nineteen days of testosterone treatment, the hormone was stopped and the epinephrine tests were continued. As can be seen, by the fortieth day a normal response to epinephrine was obtained and this was again found on the forty-eighth day. Thus, the inhibition of ACTH production occurring during testosterone therapy seems to be reversible, and normal function was regained in this patient by the seventeenth day after testosterone was omitted.

Failure to produce inhibition of the epinephrine response was seen in 4 patients. The most striking failure occurred in patient E. L. on whom repeated tests were made over a period of forty-nine days of therapy. As can be seen in Figure 4, a normal response to epinephrine was demonstrated in

this patient with the exception of tests on the forty-second and fifty-third days of study. This was seen in spite of the continuous administration of testosterone propionate for forty-six days. The smallest fall in eosinophils (16 per cent), seen on the fifty-third day, occurred following the cessation of testosterone therapy.

Relationship of epinephrine response to clinical improvement of breast carcinoma

In Table 1 are shown the results of studies carried out on 6 patients with carcinoma of the breast. The changes in epinephrine response are correlated with the clinical response of the carcinoma to testosterone recorded in the protocols. Of the breast cancer patients, 3 of the 6 showed diminished falls in eosinophils after epinephrine. These 3 improved during therapy with testosterone as indicated by relief of pain, healing or arrest of bone lesions and improved sense of well-being. The 3 patients who demonstrated repeatedly normal responses showed no clinical improvement in regard to controlling their lesions or their symptoms.

This small series is hardly conclusive but suggests that testosterone produces an effect on the pituitary which may be beneficial in providing palliation in patients suffering from breast carcinoma. That this effect is neither complete nor consistent can be seen in patient K. B. (Fig. 3) in whom inhibition of response to epinephrine during testosterone therapy occurred in the majority of tests. However, on the thirteenth day and again on the twenty-fourth day, normal responses of 50 and 65 per cent falls in eosinophil counts occurred, which suggest that pituitary inhibition is not complete.

DISCUSSION

As has been predicted from the observations of previous investigators (6, 7, 11) the release of pituitary ACTH appears to be inhibited by the administration of testosterone to human subjects. Our results indicate that this inhibition is neither complete nor constant; but 6 of 10 patients studied demonstrated a decreased response to a stimulus of constant magnitude while receiving testosterone. This is in contrast to the persistently normal response observed in 2 control patients who did not receive testosterone (Fig. 5), and indicates an inhibitory effect of testosterone on pituitary activity.

The occasional episodes of normal response to epinephrine observed in patients J. B. and K. B. who otherwise demonstrated reduction in pituitary function during testosterone therapy are difficult to explain. These episodes of "escape" from testosterone inhibition of pituitary function may be due to several mechanisms. It is possible that an additive action takes

place in which extraneous stress plus the administration of epinephrine produces a normal pituitary-adrenal cortex response. We have not, however, been able to demonstrate such factors as excitement, trauma or hemorrhage in these patients, which could be considered an extraneous stress —with the possible exception of J. B. In this patient the administration of x-ray to the pelvis for carcinoma of the uterus may have been a source of extraneous stress; but as noted previously, the first period of subnormal falls in eosinophils may have been due to eosinopenia and the second period of subnormal responses (days 20 to 34) was probably the only period of

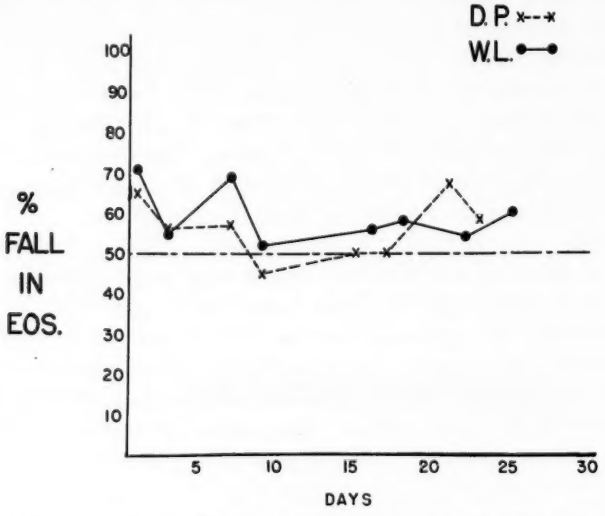

FIG. 5. Control studies on patients receiving no testosterone.
D. P.—Arrested minimal tuberculosis
W. L.—Convalescent fracture of femur

valid testosterone effect. Sayers and Sayers (8) have found that increased stress increases the amount of steroid hormone required to inhibit pituitary ACTH production. It is possible that a similar mechanism was active in our studies. Catchpole *et al.* (15) also found episodes of "escape" in their studies of the effect of testosterone in suppressing gonadotropin secretion. The appearance of these normal responses in our patients, who otherwise showed subnormal results in a majority of epinephrine tests, suggests that uncontrolled factors are active.

The possibility of the development of epinephrine tolerance must be considered in analyzing the results observed. It is possible that instead of demonstrating the effect of testosterone we have observed the diminishing

response to epinephrine alone when given repeatedly. However, in 2 patients not given testosterone, repeated epinephrine tests over period of twenty-four and twenty-six days respectively yielded consistently normal results (Fig. 5). In addition, if this were but a diminishing response to epinephrine, it would be difficult to explain the subnormal falls observed in patients F. P. and R. M., in whom the first tests were performed after testosterone had been initiated. Although no control studies in these patients were carried out, in these 2, progressive diminution in eosinophil falls may be interpreted as an increasing pituitary inhibition. To eliminate the possibility of tachyphylaxis our tests were carried out at intervals of from two to eight days in order to allow a rest period between injections of epinephrine. It appears unlikely that epinephrine tolerance occurred when injections were given at widely separated intervals, as was done in this study. However, in spite of rest periods for as long as eight days between epinephrine tests, it was observed that the fall in eosinophils was still subnormal in subjects demonstrating a change in response after testosterone. In addition, in 1 patient in whom testosterone therapy was stopped and eosinophil tests were continued, recovery of normal response to epinephrine and a subsequent greater than 50 per cent fall in eosinophils was observed. In consideration of these results, we believe that epinephrine tolerance was minimal and that the observations are primarily due to the action of testosterone on pituitary function.

We have observed that the administration of insulin creates a normal fall in eosinophils even in patients who do not show a normal response following epinephrine. Whether this indicates a difference in pathway of action or a quantitatively greater stress following insulin is not clear. Gellhorn and Frank (17) have shown that the administration of insulin produced adrenal cortical stimulation and lymphopenia even though the secretion of adrenalin in response to insulin was prevented by removing the adrenal medulla in rats. It has been stated (18) that ACTH is essential for stimulation of the adrenal cortex but there is evidence that dependence of the adrenal cortex upon pituitary activity following insulin is subject to variation in different species. Pigeons' adrenals for example appear to possess functional activity after insulin, which is not mediated by the anterior pituitary (19). In view of these observations, the present data indicate the possibility that insulin may produce adrenal cortical secretion without pituitary stimulation. Thus there may be a difference in the pathway of adrenal cortical stimulation produced by insulin and by epinephrine. Laragh and Almy, however, were unable to demonstrate adrenal cortical response in patients with hypopituitarism who were receiving insulin, so that this explanation may be untenable. It is possible also that quantitatively the stress produced by insulin is greater than that produced

by the dosage of epinephrine which we have used. Regardless of the findings with insulin administration, a significant number of patients in this study have demonstrated a reduction in response to a constant dose of epinephrine during testosterone therapy. This indicates a change in the pituitary's ability to respond to a repeated stimulus of the same order. That this phenomenon is pituitary and not adrenal in origin is indicated by the fact that parenteral ACTH produced a normal fall in eosinophils during periods of subnormal epinephrine response.

The correlation of clinical improvement and diminished response to epinephrine seen in the patients with carcinoma of the breast may be coincidental in this small series of 6 cases. The findings in the clinical failures, however, may indicate cases in which the pituitary was resistant or the dosage of androgen used was insufficient to produce a pituitary effect (12.5 to 50 mg. of testosterone propionate daily). Unfortunately no gonadotropin studies were carried out and we are therefore unable to conclude that other functions of the pituitary were affected in the subjects who showed a change in the pituitary-adrenal relationship and who improved clinically; nor are we able to conclude that gonadotropin and corticotropin are simultaneously affected. In regard to those patients who showed no change in response to epinephrine and who failed to improve clinically, continuation of the study might have altered our findings. Since the time required to produce pituitary inhibition varies from patient to patient, the continuation of testosterone and further epinephrine tests might have produced changes similar to those obtained in the patients who improved. It is tempting to consider the possibility that the improvement seen under testosterone therapy is due to a pituitary effect produced by this androgen but extension of these studies must be carried out before conclusions can be drawn.

SUMMARY AND CONCLUSIONS

Pituitary-adrenal cortex function has been studied in 10 patients receiving testosterone propionate, using the method of determining eosinophil destruction following stimulation of the pituitary by epinephrine. Six of these patients were suffering from advanced metastatic carcinoma of the breast.

Epinephrine injections and eosinophil counts were performed repeatedly on 2 other subjects studied as controls without being given testosterone. These subjects had consistently normal falls in eosinophils of 50 per cent or greater following epinephrine.

Of the patients receiving androgen, subnormal responses to epinephrine developed in 6, but in 4 others a normal response was maintained. In 1 patient who showed subnormal responses, cessation of testosterone therapy was followed by a return of normal eosinophil falls.

Two patients who reacted subnormally to epinephrine during androgen therapy were given ACTH parenterally, which yielded normal eosinophil destruction. Insulin given intravenously to these subjects was also followed by normal falls in eosinophils.

Occasional episodes of normal reaction to epinephrine with 50 per cent or greater eosinophil decreases were observed in 2 patients who otherwise showed subnormal response during androgen therapy.

In a small series of 6 breast cancer patients, subnormal eosinophil responses to epinephrine developed in 3, who showed clinical improvement. Three others also receiving testosterone showed consistently normal responses and no clinical improvement.

These studies suggest that testosterone, in the doses administered, produced suppression of the pituitary-adrenal cortex mechanism in 6 of the 10 patients studied. The effect appears to be due to reduced pituitary ACTH production in response to epinephrine stimulation rather than to diminished adrenal cortical response to ACTH. In our series of 6 cases of advanced breast cancer, diminished pituitary-ACTH function has been found to parallel clinical improvement. This finding may not be causal in relationship but may reflect a coincidental reduction in other pituitary functions more directly related to the control of breast cancer.

Acknowledgment

The authors wish to express their gratitude to Drs. Stanley E. Alderson, Crawford J. Campbell, and Arthur J. Wallingford, and the Surgical Service of the Albany Hospital for permission to study cases under their care.

PROTOCOLS OF PATIENTS WITH BREAST CANCER

Patients receiving palliation:

F. P., a 62-year-old colored female, menopausal at age 49, entered with a metastasis from carcinoma simplex in the left femur in November, 1948. Testosterone therapy (300 mg. weekly) was given from November 1948 through February 18, 1949 with relief of pain and arrest of bone metastasis. She was readmitted March 2, 1949 for pain in the lumbar region. *Therapy and course:* Received testosterone, 50 mg. daily from March 3 to 7, and 100 mg. daily from March 8 to 18, 1949. This was followed by increased appetite and decreased pain during treatment. Subsequent x-ray therapy to the femur resulted in recalcification. A third course of testosterone in September 1949 again gave relief of symptoms.

R. M., a 55-year-old white female, menopausal at age 35, had had a left mastectomy for carcinoma simplex two years before entry. One month before entry, she had back pain and metastases to lumbar vertebrae, sacrum, ribs and skull. *Therapy and course:* Received testosterone, 50 mg. per day, from March 2 to 15, with marked relief of pain; and 300 mg./week from March 15 to July 15, 1949, with complete recalcification of all bone lesions.

K. B., a 44-year-old white female, premenopausal, had had a right mastectomy performed in March 1948 for duct cell carcinoma. Back pain began two months before her October 1949 entry. There were metastases to ribs, left scapula, cervical and thoracic

vertebrae, ischium and ilium. *Therapy and course:* A dose of 50 mg. of testosterone was given daily from October 21 to November 15, 1949; then 300 mg. weekly. Pain relief, increased sense of well-being and weight gain occurred, but no improvement was noted in bone lesions. The patient died three months after therapy was started.

Patients receiving no palliation:

I. G., a 77-year-old white female, menopausal at age 40, had had a left mastectomy in May 1948. Pain in the left ribs was noted in September 1948. Cough, dyspnea, weight loss, anorexia, headache and pain in the pelvic region followed and admission occurred April 1949. X-ray examination revealed increased density in the lower portion of the upper lobe of the right lung and atrophic changes in the lumbar spine and pelvis; the ribs and skull did not show any metastatic areas in April 1949. Serum alkaline phosphatase was 2.6 millimol units; calcium, 11.3 mg. per cent; phosphorus, 2.3 mg. per cent; and total protein, 6.2 Gm. per cent. *Therapy and course:* Received testosterone 50 mg. daily for eleven days, with moderate relief of pain but marked disorientation. She was discharged to a nursing home but died several weeks later.

E. L., a 55-year-old white female, had had a hysterectomy and unilateral oophorectomy in November 1948, and a right mastectomy for scirrhous carcinoma simplex in May 1949. She entered in September 1949 with a nodular, ulcerated right chest lesion. No bone or lung lesions were found on x-ray examination. *Therapy and course:* Received testosterone 50 mg. daily for forty-six days with no change in the lesion; but increased pain and dyspnea occurred.

E. M., a 49-year-old white female, had had a bilateral salpingo-oophorectomy at age 42. Swelling and induration of the breasts was first noticed six months before entry. Pain in breasts, spine and legs occurred three months before admission. Biopsy in October 1949 showed anaplastic, desmoplastic carcinoma in the skin of the breast. Both breasts were indurated and erythematous, with bilateral axillary nodes. The liver was palpable and metastases were noted in the right femur, the ramus of the ischium on the right, the ribs and thoracic spine. *Therapy and course:* Received 12.5 mg. of testosterone daily from October 1 through October 21; and 25 mg. daily from October 22 through October 26, 1949. No improvement in symptoms or metastases occurred and the patient died October 27, 1949. Autopsy confirmed clinical evidence of widespread metastases.

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PITUITARY-ADRENAL RELATIONSHIP IN FULL-TERM AND IN PREMATURE INFANTS, AS EVIDENCED BY EOSINOPHIL RESPONSE*

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EXPOSURE of the organism to stress or the administration of a toxic substance results in the elaboration of adrenocorticotropic hormone (ACTH) by the pituitary, which in turn stimulates the adrenal cortex. This phenomenon was originally postulated by Selye (1) and demonstrated more clearly by Long (1947, (2)), Sayers and Sayers (1949, (3)) and others.

One of the manifestations of increased adrenocortical secretion is a decline in circulating eosinophils (Dalton and Selye, 1937, (4); and Thorn *et al.*, 1948, (5)). In a patient with intact adrenals, the administration of ACTH causes a pronounced decline in circulating eosinophils. In the primary hypoadrenal state, however, only the injection of one of the 11-oxygen corticosteroids (compounds E and F) will produce an identical response (Hills *et al.*, 1949, (6)).

The administration of epinephrine, acting as a nonspecific stress, results in a fall in circulating eosinophils in the presence of an intact pituitary-adrenal axis. If the integrity of this system is broken at any point, it is found that no response is obtained when epinephrine is given. ACTH will cause a decline in eosinophils only in the presence of responsive adrenal tissue (Recant, Hume, Forsham and Thorn, 1950, (7)).

Jailer (8, 9) has shown that the newborn rat will not respond to a singly applied stress until it attains an age of at least 8 days. It was further demonstrated that the newborn rat adrenal is capable of responding to exogenous ACTH as early as the first day of life. Therefore, the point of nonreactivity would appear to be within the sphere of the pituitary. It was pointed out in this study that the newborn rat was much more of a fetus than its human counterpart.[1]

Received for publication July 10, 1950.

* Aided by grants from the American Cancer Society, recommended by the Committee on Growth, National Research Council.

[1] After the completion of this manuscript, a paper by White and Sutton (Pediatrics **5**: 876, 1950) appeared which reported that term infants and older children respond in the

Consequently, it was decided to ascertain whether the same phenomenon occurs in the newborn infant, premature or term. Clinically, it has been known that the newborn infant cannot tolerate stress, *e.g.*, infection or dehydration, as well as the older child or adult.

METHOD

Two groups of newborn infants were studied, the normal full-term and the premature infants. The percentage fall in circulating eosinophils after administration of epinephrine and ACTH[2] was the method employed, due to its simplicity and feasibility in newborn infants.

a. Forty-two *normal infants* were chosen at random in their first 24 hours of life, in many instances before the first feeding was instituted. Their weight varied between 2,820 Gm. (5 lb. 14 oz.) and 4,740 Gm. (10 lb. 14 oz.) and their maturity by date ranged from 38 to 42 weeks. With blood obtained from a puncture wound in the heel, a preliminary eosinophil count was performed according to the method of Dunger (10) described by Thorn *et al.* (5). Epinephrine (1 in 1000) was given hypodermically in a single dose of 0.05 cc. and eosinophil counts were repeated two and four hours thereafter. A 40 per cent decrease was arbitrarily chosen as a positive response. If a significant fall in eosinophil count was not obtained, the test was repeated the next day. After two negative reactions to epinephrine, ACTH was administered on the third day. Intramuscular administration of this substance in a 6 mg. dose was followed by no untoward general reaction apart from blanching of the skin, which may last for several hours. This was probably due to the action of contaminants from the posterior pituitary gland.

b. Premature infants. Twenty-three premature infants of maturity ranging from 28 to 35 weeks by date and weighing between 1,120 Gm. (2 lb. 5⅓ oz.) and 2,500 Gm. (5 lb. 3⅓ oz.) at the time of the first test were studied as soon after birth as possible. The same routine as for full-term infants was followed. The infants who did not respond on the initial test were followed with epinephrine tests at intervals of a few days until they showed a positive reaction. They were also treated with a single dose of 6 mg. ACTH sometime during the course of the study.

RESULTS

A. Full-term infants

Eosinophil count. Eosinophil counts in the normal full-term infants vary

same manner as adults to the administration of epinephrine in regard to a decline in circulating eosinophils. Premature infants, however, do not react until they attain a certain weight.

[2] Kindly supplied by Dr. John R. Mote, Armour Laboratories, Chicago.

as widely as in adults. Fifty counts taken from infants 1 to 7 days old showed a wide range between 20 to 537 per cubic millimeter with a mean value of 187 per cubic millimeter. Obviously, for our purpose the initial count is not as important as the percentage drop following epinephrine or ACTH injection. A day-to-day count in 12 infants showed no significant tendency towards a rise or fall of circulating eosinophils in the first week of extra-uterine life.

Epinephrine tests. Of 42 infants tested between 4 and 24 hours of age, 23 gave a positive reaction to epinephrine administration, and 5 more showed a significant response when the test was repeated on the second day. Of the remaining 14 infants who failed repeatedly to respond to epinephrine, 11 were tested with 6 mg. of ACTH the third day. A fall of from 40 to 77 per cent in the number of circulating eosinophils was obtained in 7 infants; the remainder showed either an equivocal reaction or a negative response (Fig. 1). Unfortunately, most infants are discharged from the nursery on the fourth or fifth day of life, so that it was impossible to ascertain when the non-reactors would finally respond.

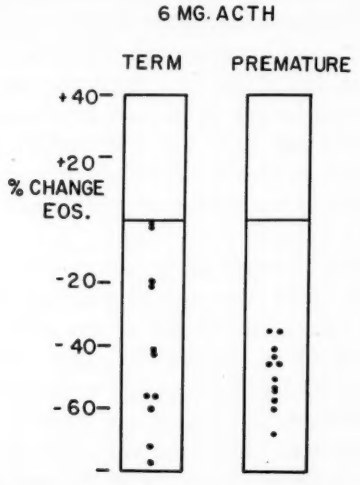

FIG. 1. The effect of 6 mg. of ACTH on the percentage change in circulating eosinophils at four hours after the injection.

B. Premature infants

Twenty-three premature infants were studied. In this group of infants, it was not possible to start the investigation on the first day of life, due to the fact that many of them were born in poor condition and others were first admitted into the nursery when they were several days old.

In no instance was there a fall in eosinophils of 40 per cent or more four

hours after the administration of 0.05 mg. epinephrine until the ninth day of life. In 1 case, a 38 per cent fall was achieved on the second day of life. In 3 infants, the decline in circulating eosinophils at two hours was greater than at four, so that the response could be considered positive at two hours. In 1 of these premature infants, a two-hour positive response oc-

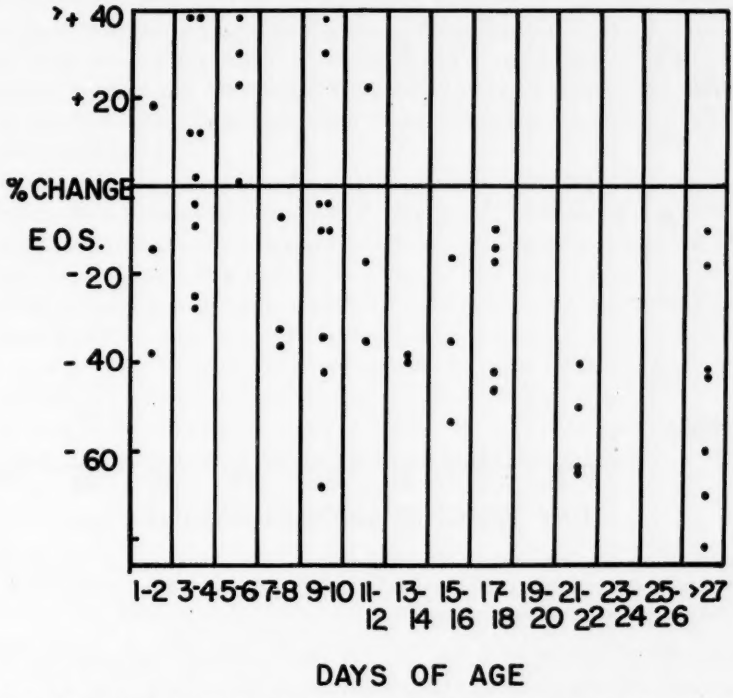

FIG. 2. Percentage change in circulating eosinophils four hours after the administration of 0.05 mg. of epinephrine to premature infants. The abscissa represents age in days, and the ordinate, the percentage change in eosinophils. These data represent all epinephrine tests performed.

curred on the ninth day. In Figure 2, the percentage alteration in eosinophil count at four hours after the administration of epinephrine is plotted against the age of the infant. It is evident that, as the infant grows older, the percentage of positive results increases.

In general, the smaller the birth weight, the longer it took for the result of the epinephrine test to become positive. For example, infant R.A. weighed 920 grams at birth and the epinephrine reaction first became positive at 35 days of age. Infant A.T.'s birth weight was 1,040 grams, and the administration of epinephrine resulted in but a 19 per cent decrease in eosinophils at 41 days of age. The patient was discharged before the re-

sponse became positive. Infant G.I. first responded at 22 days; its birth weight was 1,120 grams. There were, of course, exceptions, but this relationship appeared to be the general rule (Fig. 3).

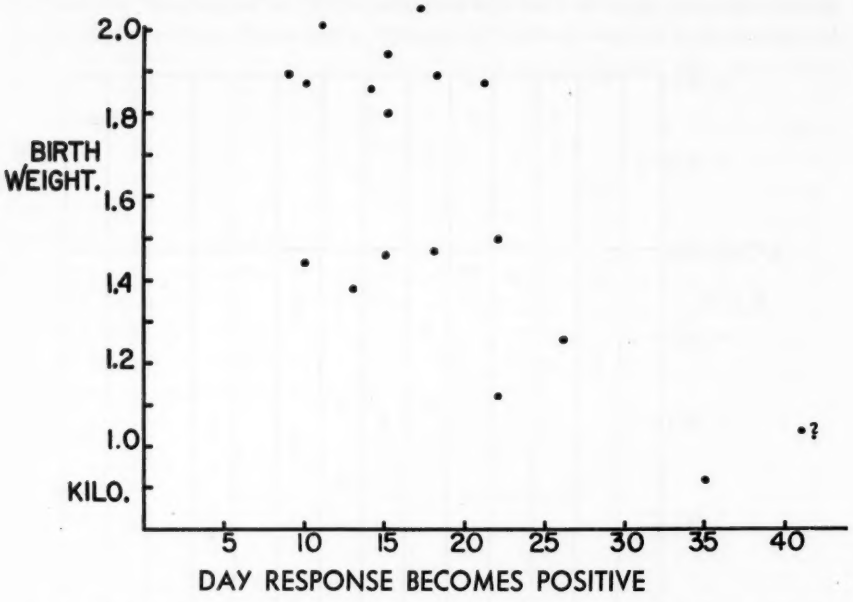

FIG. 3. Ordinate represents birth weight and abscissa the day at which the result of the epinephrine test becomes positive. ?= 19 per cent fall in eosinophils, but infant was discharged on following day.

The administration of 6 mg. ACTH resulted in an appreciable fall in circulating eosinophils in all the premature infants tested (Fig. 1).

DISCUSSION

The results obtained on the newborn human infant are somewhat similar to those in the rat, if one takes into consideration the difference in the state of maturity at birth in the two species. The newborn rat at birth is poikilothermic, its eyes and ears are closed and it is devoid of fur. Consequently, the premature human infant is more nearly similar to it in degree of maturation.

The prematurely-born human infant does not respond by a significant decrease in circulating eosinophils to a single injection of 0.05 mg. of aqueous epinephrine until it reaches from 9 to more than 41 days of age.[3] It appears

[3] These results are very similar to those presented by White and Sutton. The latter authors, however, did not locate the point of nonreactivity in the pituitary-adrenal system since they did not test the response to exogenous ACTH.

that the more immature at birth, the longer the period of insensitivity. In the group of infants born at term, there was at least a 40 per cent decline in circulating eosinophils in two thirds of the cases. In this group, however, birth weight could not be correlated with responsiveness to epinephrine.

The adrenals of practically all the infants responded to the administration of exogenous ACTH as evidenced by a fall in circulating eosinophils. Therefore, it appears that although the adrenal cortex is histologically different from that of the adult, it responds in the same way physiologically. Newborn infants are less sensitive to ACTH than older ones (Venning (11)), which may explain why a few infants failed to respond to the 6 mg. dosage employed.

Venning (12) has recently presented evidence of increased glucocorticoid excretion in a premature infant with pulmonary atelectasis and has concluded that the premature newborn can respond to stress. However, this is a stress which is probably greater in intensity and more sustained than one injection of 0.05 mg. of epinephrine. Recently, in our laboratory, we have adduced evidence that the infant rat will also respond to a sustained stress over a period of five days. The explanation appears to lie in the fact that the pituitary responds to a single stress by liberating the ACTH that it has stored or has in reserve. (Accordingly, no ACTH can be detected by bioassay in newborn rats.) Repeated stress causes the pituitary to synthesize more ACTH than it does normally, and this in turn becomes available for liberation, if the stress is maintained.

There is no evidence of adrenal insufficiency in the premature or term infants as evidenced by the urinary excretion of corticoids. This has been observed by Venning (12), Day (13) and King and Mason (14). The lack of response to a singly applied stress in the premature and some term infants may possibly be due to the same mechanism as obtains in the rat.

The maintenance of homeostasis in the organism depends upon a multitude of mechanisms. In view of the data presented, it would appear that in premature human infants, the mechanism for adjustment toward stress is not completely developed at birth, but requires a variable length of time for its complete attainment. These observations are not without precedent. McCance and Young (15) and Heller (16) have demonstrated that rigid fluid restriction in newborn humans and rats does not result in the excretion of a urine of higher specific gravity and osmolar concentration as does such a procedure in an older individual. Thus, the renal tubules are not capable of reabsorbing water in response to a dehydrating stimulus. This is an example of another homeostatic mechanism not fully developed at birth. Numerous other mechanisms are not completely functional at birth. Both the premature infant and newborn rat have great difficulty in maintaining body temperature in response to changes in environmental

temperature. Consequently, the hazards of prematurity are due to the inability of the organism to regulate certain aspects of its "milieur interieur."

SUMMARY

Administration of epinephrine to premature infants does not bring about a significant fall in the number of circulating eosinophils before the ninth day of age, whereas the majority of full-term infants react promptly in the first 24 hours of life.

In general, the less the birth weight, the greater the interval before the result of the epinephrine test becomes positive.

Administration of ACTH to both types of infants results in most instances in a significant fall of eosinophils as early as the second or third day of life.

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ADRENAL CORTICAL CHANGES IN RATS WITH VARIOUS TYPES OF EXPERIMENTAL HYPERTENSION*

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THE functional and general pathologic changes occurring in rats with experimental hypertension following 1) encapsulation of the kidney, 2) the administration of desoxycorticosterone acetate, or 3) the injection of anterior pituitary preparations, have been reported elsewhere (Masson *et al.* (1, 2)). The present paper deals with the concurrent histologic and histochemical alterations in the adrenal cortex.

Interest in the adrenal changes was initially aroused by Selye's (3) speculation that anterior pituitary preparations induce hypertension by stimulating the release of salt-regulating hormones from the adrenal cortex. Secondly, evidence has appeared that adrenal changes accompany renal damage and experimental renal hypertension. Thus, Olson and Deane (4) observed evidence of increased secretion of salt-regulating hormones in weanling rats with kidney damage from choline deficiency. And a survey (Masson, Corcoran and Page, unpublished) of adrenal weights in 100 rats with hypertension of renal origin showed that the paired adrenal glands were larger (av. wt., 63 mg.) in rats with severe hypertension than in those with hypertension of moderate degree (av. wt., 47 mg.).

The present study describes the histologic and histochemical responses occurring in the adrenal cortex of rats made hypertensive by each of the three different methods mentioned above. The outer portion of the cortex, the glomerulosa, received the greatest attention, since this is the zone believed to secrete salt-regulating hormones (5). The effects on the adrenal cortex of the injection of kidney extracts containing renin were also investigated.

MATERIALS AND METHODS

Adult rats of the Sprague-Dawley and Wistar albino strains were used. Except in experiment 3, they were fed Purina dog chow.

Received for publication July 12, 1950.

* This study was supported in part by grants to the Department of Anatomy, Harvard Medical School, from the American Cancer Society, 1) on the recommendation of the Committee on Growth of the National Research Council, and 2) from an Institutional grant to Harvard University; and one to the Cleveland Clinic Foundation from the United States Public Health Service (National Heart Council).

1. *Rats with wrapped kidneys (silk perinephritis).* Male rats of the Sprague-Dawley strain were divided into 2 control and 2 experimental groups (Table 1). The control groups consisted of 4 intact and 4 unilaterally nephrectomized rats, all of which were given tap water to drink. The first group of experimental rats had both kidneys wrapped with silk cloth (Page (6)); these rats were supplied with tap water. In the second group, the rats had only one kidney wrapped and were given a 1 per cent solution of sodium chloride to drink.

2. *Rats treated with desoxycorticosterone or with compound S.* Female rats of the Sprague-Dawley strain were divided into 7 groups (Table 2). The rats in groups 1, 2, 5, 6 and 7 were unilaterally nephrectomized; all but those in group 4 received 1 per cent saline to drink. Group 1 consisted of 6 control animals. Groups 2 and 3 received a daily subcutaneous injection of 2.5 mg. of desoxycorticosterone acetate (DCA) in water suspension; in addition, the rats in group 3 had one kidney wrapped. The rats in group 4 had both kidneys wrapped with silk which was coated with DCA at the time of operation. These animals received tap water to drink.

Group 5 served as controls for groups 6 and 7. The rats in group 6 had subcutaneous implantations of two 30-mg. pellets of DCA; those of group 7, two pellets of 17-hydroxy-11-desoxycorticosterone (compound S) acetate. The latter steroid possesses a slight salt-retaining activity (7).

3. *Rats treated with anterior pituitary powder.* Female Sprague-Dawley rats were unilaterally nephrectomized and divided into 3 groups (Table 3). All of these animals were fed a 30 per cent protein diet and were given 1 per cent sodium chloride to drink. Group 1 received no further treatment. The rats in groups 2 and 3 received injections of a suspension of anterior pituitary powder (APP). This was a lyophilized powder (purchased from Des-Bergers Laboratories, Ltd., Montreal) suspended in physiologic saline containing 10 per cent alcohol. A daily dose of 40 mg. was given in 2 subcutaneous injections. The rats in group 3 received in addition a daily injection of 2.5 mg. of DCA in aqueous suspension.

4. *Rats injected with partially purified renin.* Female rats of the Wistar strain were divided into 4 groups of 6 each (Table 4). All were unilaterally nephrectomized and received 1 per cent saline to drink. Group 1 served as controls. The rats in group 2 received 2 subcutaneous injections daily of 0.25 cc. of stock purified renin. The stock renin solution, prepared from hog kidneys by Dr. A. A. Green, contained 30 mg. of protein per cc; 0.1 cc. produced a 35-mm.-Hg rise in blood pressure in a 10 Kg. dog under Nembutal anesthesia. Group 3 received one tenth of the dose given to group 2. This was prepared by diluting the stock solution with 1 per cent saline so that the quantity of fluid injected was the same. Group 4 received

a kidney extract containing the same amount of protein per cc. as the stock renin but only one tenth of its pressor activity.

Blood pressure determinations were made regularly throughout the experiments by plethysmographic methods (8, 9). With the exception mentioned below, each rat was placed unanesthetized in a warm box at 45°C. for three minutes before the blood pressure was read. The rats in experiment 2, groups 5 to 7, were placed instead in a warm room at 30°C. for one hour before recording their blood pressures; this treatment resulted in somewhat higher readings than the warm-box method.

The animals were killed by bleeding. The adrenal glands were dissected free of fat and fixed in 10 per cent formalin or in Zenker's fluid. The glands were weighed after fixation on a Roller-Smith torsion balance.

Formalin-fixed glands from each group of animals were sectioned at 15 micra on a freezing microtome. From each of the glands one section was stained with a 1 per cent solution of sudan black B in 70 per cent alcohol, one by the Ashbel-Seligman (10) method for ketonic steroids, and one by the Schultz method for "cholesterol." Two sections were mounted unstained, one of which was untreated and one of which was first extracted for one hour in cold acetone. These latter two sections were compared under the polarizing and fluorescence microscopes for the presence of acetone-soluble birefringent and fluorescent substances. These methods, as a group, are believed to localize the sites of formation of steroid hormones; and they also permit an evaluation of secretory activity (5).

Other formalin-fixed glands and those fixed in Zenker's fluid were embedded in paraffin, sectioned at 10 micra and stained with Ehrlich's hematoxylin and eosin.

The width of the zona glomerulosa was measured on the frozen sections stained with sudan black or on the paraffin sections. For this, camera lucida outlines of the glomerulosa were made at 4 different points around the gland at a magnification of 720 ×. These 4 readings were averaged and converted to micra.

OBSERVATIONS

Control groups. The data for the control rats are tabulated with those for the appropriate experimental groups. The paired adrenal glands of large, untreated male rats weighed about 12 mg. per 100 Gm. of body weight (Table 1, group 1). In these animals, the blood pressure averaged 104 mm. Hg. Unilateral nephrectomy alone raised the blood pressure slightly, but did not affect the weight of the adrenals (group 2). When 1 per cent sodium chloride was added to the drinking water for rats which had been unilaterally nephrectomized, there was a marked increase in the relative weights of the glands (Tables 2, 3, and 4). It is to be noted, how-

ever, that these saline-treated rats were females and also weighed less. The adrenals are relatively larger in small rats, and also larger in females than in males (11).

In the untreated rats, the zona glomerulosa appeared moderately fatty (Fig. 1) and was approximately 40 micra in width. Unilateral nephrectomy alone increased the width of the zone slightly (45 micra). When saline was supplied, the zone was narrower: after ten days (Table 4) it still retained considerable amounts of lipid (Fig. 2), but by twenty to thirty days

TABLE 1. THE EFFECTS OF ENCAPSULATING THE KIDNEYS OF RATS

Group	Treatment	No. of rats	Duration in days	Body weight, Gm.		Blood pressure,* mm. Hg	Adrenals, mg./100 Gm. body wt.	Glomerulosa width, micra
				Initial	Final			
1	none	4	41	170	265	104 (98–110)	11.9	39
2	unilateral nephrectomy	4	41	180	268	115 (108–130)	11.7	45
3	both kidneys wrapped	4	73	110	219	158 (138–180)	27.0	77
		1	95	139	257	143 (130–152)	18.2	70
		1	120	120	154	190 (180–202)	41.8	115
4	one kidney wrapped, NaCl	3	30	165	195	156 (145–162)	16.9	70
		4	37	167	206	145 (124–172)	16.1	61

* Average of the last 3 blood pressure determinations.

(Tables 2 and 3) it was almost completely depleted of fatty inclusions (Fig. 3). In all of the control rats, the fasciculata was broad and contained more lipid in its outer portion than further inward (Figs. 12 and 13). Whenever lipid occurred in the glomerulosa, the 2 zones were separated by a fat-free transitional zone. In both the glomerulosa and fasciculata, the lipid droplets displayed the tests characteristic of ketosteroids—the Ashbel-Seligman carbonyl reaction, a yellowish-green fluorescence, birefringence, and the Schultz reaction. The birefringent crystals in the glomerulosa of the normal rats were moderately crowded and mixed in size.

TABLE 2. THE EFFECTS OF DESOXYCORTICOSTERONE AND OF COMPOUND S ON RATS WITH AND WITHOUT ENCAPSULATED KIDNEYS

(All of the rats except those in group 4 received saline as drinking water. The animals in groups 1, 2, 5, 6 and 7 were unilaterally nephrectomized as well.)

Group	Treatment	No. of rats	Duration in days	Body weight, Gm.		Blood pressure,* mg. Hg	Adrenals, mg./100 Gm. body wt.	Glomerulosa, width, micra
				Initial	Final			
1	none	6	22	121	154	111 (102–125)	32.1	30
2	DCA, 2.5 mg. daily	2	40	117	94	196 (185–200)	53.5	25
		1	47	97	130	170 (180–170)	34.8	—
		1	111	150	220	190 (180–205)	30.7	35
3	DCA, 2.5 mg. daily; 1 kidney wrapped	3	30	153	177	176 (170–186)	17.1	25
4	Both kidneys wrapped;	2	57	150	247	112 (91–125)	20.7	42
	DCA on wrappings	3	86	86	188	141 (108–155)	28.3	38
5**	none	5	30	110	197	139 (115–142)	23.8	31
6**	DCA, two 30-mg. pellets	4	30	97	126	194 (175–208)	25.7	28
7**	Compound S, two 30-mg. pellets	4	30	100	170	135 (125–150)	23.9	31

* Average of the last 3 blood pressure determinations.

** The rats in groups 5 to 7 were kept in a warm room for one hour before blood pressure readings were made.

Encapsulation of the kidneys (Table 1). Rats with both kidneys wrapped with silk (silk perinephritis) displayed a variable rise in blood pressure and a relative enlargement of the adrenal glands. The adrenals were largest (41.8 mg. per cent) in a rat with an average blood pressure of 190 mm. Hg.

In all of these rats the zona glomerulosa was enlarged and laden with small lipid droplets (Figs. 4 and 5). The droplets contained uniformly fine birefringent crystals. The fasciculata appeared enlarged and its lipid content was somewhat increased (Fig. 15), as observed in conditions of moderate stress.

Rats having one kidney wrapped and receiving saline (group 4) showed approximately the same rise in blood pressure and the same enlargement of the glomerulosa (Fig. 6). The fasciculata was not broader than normal in these rats, however.

Desoxycorticosterone and compound S (Table 2). The daily injection of desoxycorticosterone acetate (group 2) or the implantation of DCA pellets (group 6) in unilaterally nephrectomized rats given saline caused a marked rise in blood pressure. The relative adrenal weights were increased

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PLATE 1

Frozen sections of the adrenal cortex stained with sudan black B and photographed with a blue filter (Wratten H). ×200. Wherever the transition from glomerulosa to fasciculata is difficult to see, a line has been drawn in on the righthand side.

FIG. 1. Control rat, intact, tap water (Table 1, group 1). The zona glomerulosa measures 39μ and is unevenly filled with lipid. A transitional zone separates the glomerulosa from the fasciculata.

FIG. 2. Control rat, unilaterally nephrectomized and saline, 10 days (Table 4, group 1). The glomerulosa is narrow but contains moderate amounts of fat.

FIG. 3. Control rat, unilaterally nephrectomized, high protein diet and saline, 31 days (Table 3, group 1). The glomerulosa has shrunk to 30μ and is completely depleted of lipid.

FIG. 4. Rat with both kidneys wrapped, tap water, 73 days (Table 1, group 3). The glomerulosa measures 76μ and contains abundant lipid.

FIG. 5. Rat with both kidneys wrapped, tap water, 120 days (Table 1, group 3). The glomerulosa is still broader than in Fig. 4.

FIG. 6. Rat with one kidney wrapped and saline, 30 days (Table 1, group 4). The glomerulosa measures 70μ and appears intensely fatty.

FIG. 7. Unilaterally nephrectomized rat receiving stock renin solution and saline, 10 days (Table 4, group 2). The glomerulosa is broad; the transitional zone between the glomerulosa and fasciculata has disappeared.

FIG. 8. Unilaterally nephrectomized rat receiving 2.5 mg. of DCA and saline, 111 days (Table 2, group 2). The glomerulosa is narrow and entirely free of lipid.

FIG. 9. Rat with both kidneys wrapped, with DCA on wrappings, tap water, 20 days. The glomerulosa is narrow but quite fatty. In rats so treated for longer periods (Table 2, group 4) the zone is still narrower and somewhat depleted.

FIG. 10. Unilaterally nephrectomized rat receiving 40 mg. of anterior pituitary powder daily, high protein diet and saline, 48 days (Table 3, group 2). The glomerulosa resembles that in Fig. 3.

FIG. 11. Unilaterally nephrectomized rat receiving 40 mg. of APP and 2.5 mg. of DCA daily, high protein diet and saline, 13 days (Table 3, group 3). The glomerulosa is atrophied as in Figs. 3, 8 and 10.

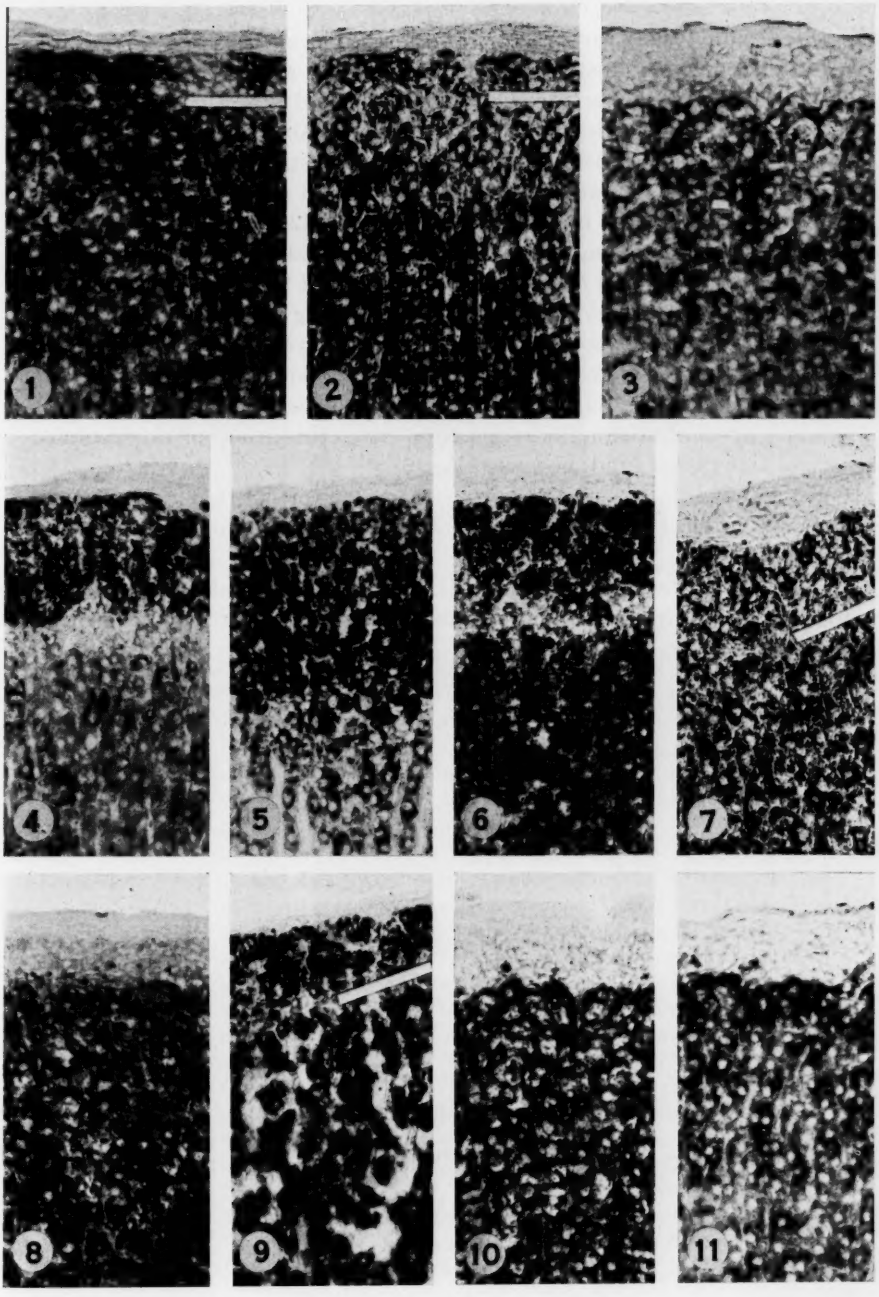

PLATE 1

in those animals with severe vascular disease. The glomerulosa was narrow and fat-free (Fig. 8), just as in the controls which received saline to drink. The fasciculata appeared unusually fatty (Fig. 14).

In 2 rats, desoxycorticosterone injections and saline administration were both discontinued after 47 and after 67 days, respectively. A slight drop in blood pressure followed (from 180 mm. Hg. at 67 days to 162 mm. at 94 days; and from 186 mm. Hg at 47 days to 142 mm. at 94 days). The width of the glomerulosa in these 2 rats was 29 and 37 micra, respectively, and the zone contained nearly normal amounts of lipid.

Wrapping one kidney of rats receiving 2.5 mg DCA daily (group 3) did not offset the atrophy characterizing desoxycorticosterone administration. Moreover, merely applying DCA to the kidney wrappings (group 4) prevented the marked enlargement of the glomerulosa noted in rats with renal encapsulation alone. The glomerulosa in this group of rats retained normal amounts of lipid, however (Fig. 9).

The administration of 17-hydroxy-11-desoxycorticosterone (compound S) failed to alter blood pressure, adrenal weight or glomerulosal width (group 7).

Anterior pituitary powder (Table 3). The daily administration of 40 mg. of anterior pituitary powder resulted in stimulation of body growth,

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PLATE 2

Frozen sections of adrenal glands stained with sudan black B and photographed with a yellow filter (Wratten G). ×65.

FIG. 12. Control rat, unilaterally nephrectomized and tap water, 41 days (Table 1, group 2). The glomerulosa is rich in lipids. A narrow transitional zone separates the glomerulosa and fasciculata. The fasciculata is of normal width and characteristically more fatty in its outer portion than further toward the medulla.

FIG. 13. Control rat, unilaterally nephrectomized, high protein diet and saline, 48 days (Table 3, group 1). The glomerulosa is narrow and free of fat. The fasciculata is slightly broader than in Fig. 12.

FIG. 14. Unilaterally nephrectomized rat receiving 2.5 mg. of DCA daily and saline, 40 days (Table 2, group 2). The glomerulosa is narrow and free of fat; the fasciculata is unusually laden with lipid (*cf.* Greep and Deane (16)).

FIG. 15. Rat with both kidneys wrapped for 95 days, tap water (Table 1, group 3). The glomerulosa is broad and irregularly filled with lipid. The fasciculata is so broad that its entire width is not shown here.

FIG. 16. Unilaterally nephrectomized rat receiving 40 mg. of anterior pituitary powder daily, high protein diet and saline, 48 days (Table 3, group 2). The glomerulosa is atrophied as in the control rat (Fig. 13). The fasciculata is markedly broader; lipid has appeared in the zona reticularis.

FIG. 17. Unilaterally nephrectomized rat, receiving 40 mg. of APP and 2.5 mg. of DCA daily, high protein diet and saline, 13 days (Table 3, group 3). The glomerulosa is completely depleted of lipids. The fasciculata is so broad that its inner border is not shown.

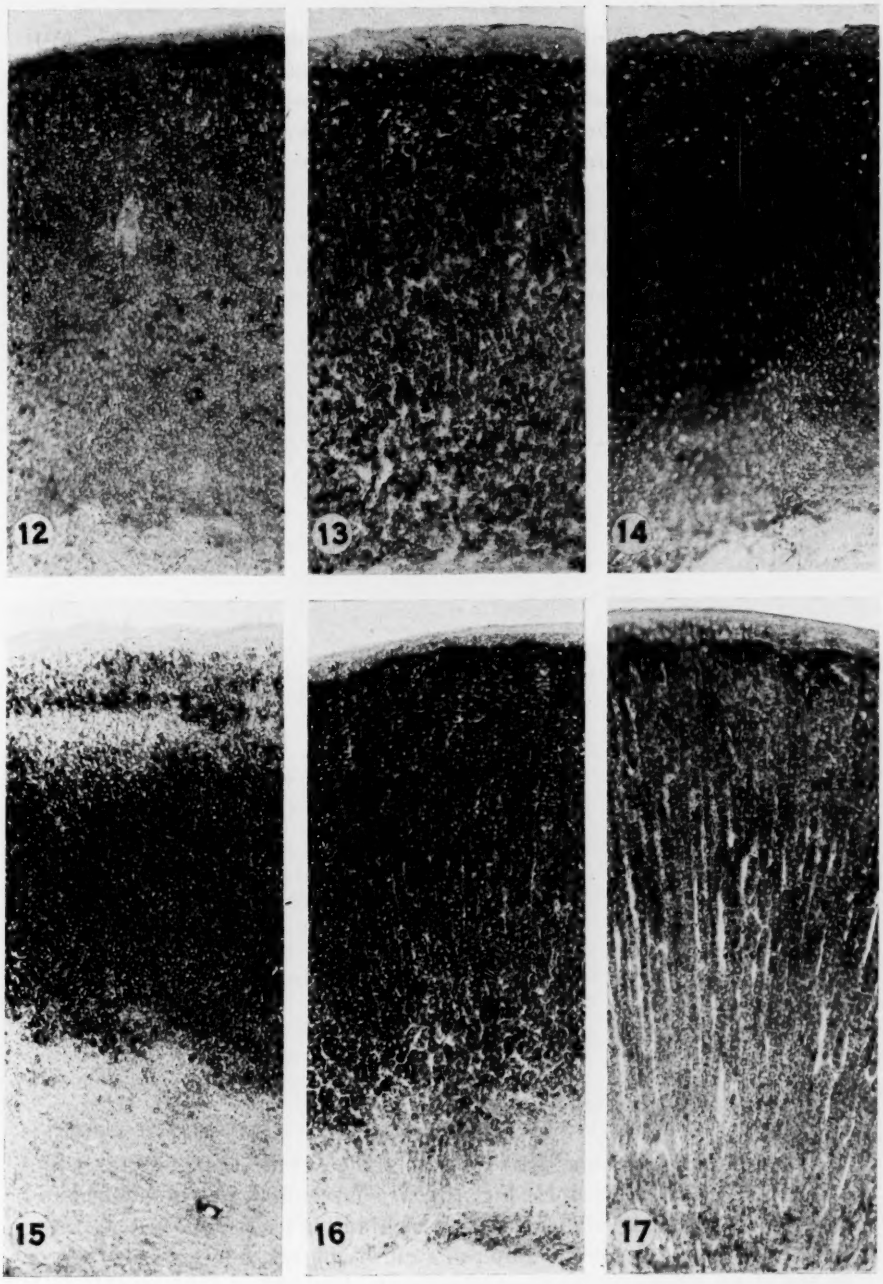

PLATE 2

especially at the beginning of treatment. Many of the rats receiving APP died during the course of treatment, and are not included in the table. The blood pressure was moderately elevated; in only the 2 which survived for forty-eight days, however, did it reach 190 mm. Hg (group 2). The adrenals of all of the rats receiving this treatment were significantly enlarged.

TABLE 3. THE EFFECTS OF ANTERIOR PITUITARY POWDER

(All of the rats were unilaterally nephrectomized and received a 30 per cent protein diet and 1 per cent saline to drink.)

Group	Treatment	No. of rats	Duration in days	Body weight, Gm.		Blood pressure,* mm. Hg	Adrenals, mg./100 Gm. body wt.	Glomerulosa width, micra
				Initial	Final			
1	none	4	26	112	143	111 (96–115)	23.4	32
		4	31	111	176	109 (98–116)	27.7	30
		4	45	118	177	103 (90–116)	26.3	32
2	APP, 40 mg. daily	2	48	102	157	174 (152–195)	61.1	25
3	APP, 40 mg. daily and DCA, 2.5 mg. daily	1	13	114	146	128	41.9	42
		3	17	115	105	147 (130–175)	81.0	41
		1	24	104	95	130 (123–138)	85.3	22
		2	29	130	161	165 (150–175)	33.6	29

* Average of the last 3 blood pressure determinations.

The glomerulosa was as narrow as in the controls supplied with saline and equally depleted of lipid (Fig. 10). The fasciculata was broadened and moderately fatty; an additional zone of lipid made its appearance in the reticularis (Fig. 16). In one gland of a surviving rat, a large necrotic area occupied an otherwise depleted fasciculata (Fig. 18).

When anterior pituitary powder and desoxycorticosterone were administered concomitantly (group 3), hypertension developed more rapidly than with APP alone. The resistance of these rats was so poor, however, and so many died that the experiment was terminated on the twenty-ninth

FIG. 18. One adrenal gland from a unilaterally nephrectomized rat receiving 40 mg. of APP daily, high protein diet and saline, 48 days (Table 3, group 2). Frozen section stained with sudan black B, ×15. This gland is extremely enlarged; a large necrotic area occupies the otherwise depleted fasciculata (*cf.* Deane and McKibbin: *Endocrinology* **38**: 385, 1946 (Fig. 12)).

day. In these animals, also, the adrenal glands were enlarged. At ten to seventeen days, the glomerulosa was of normal width or broader and contained considerable amounts of lipid. Thereafter the zone shrank and became depleted (Fig. 11). The fasciculata was exceptionally broad (Fig. 17).

Renin (Table 4). To examine the possibility that products released by the wrapped kidneys accounted for the enlargement of the glomerulosa in rats with renal hypertension, 3 groups of rats were given, for a period

of ten days, kidney extracts containing renin. Animals receiving the largest dose of renin (group 2) lost weight and displayed a marked diuresis. The blood pressure was also elevated. Their adrenals were slightly enlarged and the glomerulosa broadened (Fig. 7).

Other rats (groups 3 and 4), receiving one-tenth the dose of renin used for group 2, showed no diuresis or gross enlargement of the adrenals. The glomerulosa was wider than in the controls, however. The rats in group 4

TABLE 4. THE EFFECTS OF EXTRACTS CONTAINING RENIN

(All of the rats were unilaterally nephrectomized and received 1 per cent saline to drink.)

Group	Treatment	Dose of renin in cc.	No. of rats	Duration in days	Body weight, Gm.		Diuresis on last day, cc.	Blood pressure,* mm. Hg.	Adrenals, mg./100 Gm. body wt.	Glomerulosa width, micra
					Initial	Final				
1	none	0	6	10	104	130	18	114 (100–135)	24.7	47
2	renin	0.5	6	10	104	96	115	150 (135–162)	32.5	82
3	renin	0.05	5	10	104	127	23	139 (130–142)	24.5	57
4	kidney extract†	0.05	6	10	107	122	23	130 (120–149)	26.9	65

* Average of the last 3 blood pressure determinations.

† The protein content of the kidney extract was equal to that of the renin solution used for group 2, and 10 times higher than that used for group 3.

received the same amount of protein as those in group 2 but only 0.1 of its pressor activity and responded in the same way as those in group 3 receiving a tenfold dilution of the stock renin solution. It therefore appears that the action of these extracts on the glomerulosa was related to their renin content and not to nonspecific kidney protein.

In the rats receiving the larger dose of renin, the transitional zone was obliterated (Fig. 7) and the fasciculata was unusually laden with fat. These changes both signify increased stimulation of the adrenal cortex by the anterior pituitary gland.

DISCUSSION

The response of the adrenal cortex has been assessed in rats made hypertensive by three different procedures—encapsulation of the kidneys, administration of desoxycorticosterone acetate, and the injection of anterior

pituitary powder. The results suggest that these three treatments induce hypertension through separate physiologic pathways.

In the present experiments, the rats receiving either desoxycorticosterone or anterior pituitary powder were given 1 per cent saline to drink. Saline treatment alone induced atrophy and lipid depletion of the zona glomerulosa of the adrenal cortex, as reported by Badinez and Croxatto (12), Knowlton, Loeb, Seegal and Stoerk (13) and Bacchus (14). A similar atrophy induced by potassium deficiency was thought by Deane, Shaw and Greep to depend on the elevated sodium/potassium ratio in the blood. That is, the requirement for a salt-regulating hormone might be reduced so that the zone of formation, the glomerulosa, would undergo involution. Such atrophy has been found to occur as well in rats that receive desoxycorticosterone and are given tap water to drink (Sarason (15); Greep and Deane (16)).

The injection of anterior pituitary powder in rats receiving saline failed to stimulate the glomerulosa, although it did cause hypertrophy of the fasciculata. It would seem, therefore, that the hypertension developing in these animals cannot be attributed to augmented output of salt-regulating hormones, as postulated by Selye and since supported by others (*e.g.*, Symington and Goodall (17)). Bergner and Deane (18) could find no evidence of increased output of salt-retaining hormones in rats receiving adrenotropin. Moreover, the physiologic response in animals receiving APP differs from that in rats treated with DCA (Masson, Corcoran and Page (1)). The cause of hypertension in pituitary-treated rats remains unknown. Pituitary adrenotropin, which induces the release of 11-oxygenated corticoids, has not been found to cause renal changes in normal animals or in unilaterally nephrectomized rats fed a high sodium diet (Simpson, Li and Evans (19); Hay *et al.* (20)). Moreover, adrenal cortical extracts do not induce hypertension in normal or serum-nephritic rats (Knowlton *et al.* (21)). Knowlton *et al.* (22) found, however, that cortisone, like DCA, increases the severity of pre-existing renal hypertension. It seems possible, therefore, that the secretion of 11-oxygenated hormones by the fasciculata may augment the hypertension induced by anterior pituitary powder.

Encapsulation of the kidneys or the injection of renin each resulted in marked enlargement of the zona glomerulosa, whether or not the rats received saline. There was also a variable enlargement of the fasciculata, which probably may be attributed to the stress of the disease.

Enlargement of the glomerulosa probably signifies increased output of salt-regulating hormones, a conclusion further supported by the fact that the lipid droplets in the cells were small and the birefringent crystals fine. Such apparent stimulus of the glomerulosa has previously been encountered

only when the sodium/potassium ratio in the blood stream has decreased (Deane, Shaw and Greep (5); Nichols (23); Badinez and Croxatto (12)): but the electrolyte ratio is unchanged or even slightly increased in rats with renal hypertension (Friedman (24)). Such rats also experience polydipsia and polyuria (Oster and Martinez (25)) and tend to select a low sodium diet (Abrams *et al.* (26)). This behavior may result from the increased output of salt-regulatory steroids. In the absence of further data, the possibility is suggested that renin itself stimulates the glomerulosa. The increased secretion of salt-regulating hormones by the glomerulosa may in turn exacerbate the hypertension.

Whereas high salt intake failed to offset the hypertrophy of the glomerulosa in animals with wrapped kidneys, the injection of desoxycorticosterone completely inhibited the reaction and produced atrophy of the zone. The mechanism of atrophy in this situation remains obscure. Earlier, Greep and Deane postulated that desoxycorticosterone inhibits the zone indirectly by raising the sodium/potassium ratio in the blood. The present results point to a more specific effect, since increased sodium intake failed to have the same influence.

SUMMARY AND CONCLUSIONS

The histologic and histochemical responses of the adrenal cortex were studied in rats made hypertensive by 1) encapsulation of the kidneys with silk, 2) the administration of desoxycorticosterone acetate, or 3) the injection of anterior pituitary powder. The effects of the injection of kidney extracts containing renin were also ascertained.

Encapsulation of the kidneys was followed by marked enlargement and apparent stimulation of the zona glomerulosa of the cortex, whereas desoxycorticosterone produced a disuse atrophy, and the anterior pituitary powder caused no change in this zone.

Enlargement of the glomerulosa also followed the injection of partially purified solutions of renin.

The enlargement of the glomerulosa in experimental renal hypertension probably does not result from any upset in the electrolyte balance and may possibly be a response to renin itself.

Acknowledgments

Desoxycorticosterone acetate was generously supplied by Dr. E. Henderson of the Schering Corporation and Compound S acetate by Mr. Adrian D. Joyce of the Glidden Company.

We wish to thank Mrs. Arnolda van Dorp for her assistance in preparing the slides and evaluating the results.

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METHYLANDROSTENEDIOL: A PROTEIN-ANABOLIC STEROID WITH LITTLE ANDROGENIC ACTIVITY*†

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THE need for a compound which stimulates general tissue growth has led to examination of a number of agents by several methods of assay. The final test of clinical usefulness of such a compound is its ability to produce an increment of tissue in man as measured by weight, height, and metabolic balance studies. The hormones found to be potent in this respect are also strongly androgenic, a quality which limits their clinical application. Although the growth hormone of the anterior pituitary gland fulfills the criteria of tissue growth in animal experimentation and is devoid of androgenic activity, existing preparations of this hormone have been disappointing in man (1, 2). Previous experiments have suggested that the androgenic potency of the steroids does not necessarily parallel their anabolic activity (3). These studies have also indicated that the weight of the levator ani muscle of the castrate male rat reflects the anabolic efficacy of administered steroids. This muscle is restored to normal, or even rendered hypertrophic, by potent anabolic agents: testosterone propionate, unesterified testosterone, methyltestosterone, testosterone cyclopentylpropionate, and the pituitary growth hormone. Progesterone, the anabolic effects of which have been inconclusive, produces a moderate but significant growth of this muscle. No growth results from steroids which have been found ineffective by the metabolic balance method: estradiol dipropionate, desoxycorticosterone acetate, ethinyl testosterone, and cistestosterone. Conditions producing tissue catabolism, such as hyperthyroidism and administration of cortisone, are accompanied by a decrease in the weight of the already atrophic muscle (4).

In the search for a hormone which might stimulate tissue growth and yet not possess androgenic or estrogenic properties, we have studied the

Received for publication July 13, 1950.

* Presented at the Annual Meeting of the Association for the Study of Internal Secretions, San Francisco, California, June 23, 1950.

† Aided by grants and materials from Organon, Inc., Orange, N. J., and Ciba Pharmaceutical Products, Inc., Summit, N. J. The use of methylandrostenediol was suggested and the compound made available to us by Dr. Kenneth Wade Thompson, Organon, Inc., Orange, N. J.

effects of various steroids upon the weight of the levator ani muscle of the castrate male rat. Simultaneous examination of the seminal vesicles provided a measure of androgenic activity.

METHOD

Male rats of the Long-Evans strain were castrated at 30 days of age and treated with subcutaneously administered test material daily for one week prior to decapitation at the age of 60 days. The levator ani muscles and seminal vesicles were dissected and weighed as described elsewhere (3).

EXPERIMENTAL DATA AND DISCUSSION

The results of this experiment are tabulated in Table 1.

TABLE 1. EFFECTS OF VARIOUS HORMONES UPON THE WEIGHT OF THE LEVATOR ANI MUSCLE AND SEMINAL VESICLES OF THE CASTRATE MALE RAT

No. of rats	Group	Levator ani		Seminal vesicles, Weight in mg.
		Weight, mg.	Wet: Dry ratio	
		mean $\pm \sigma_m$		mean $\pm \sigma_m$
21	Normal	101 ± 6.3	4.5	276 ± 15.5
23	Castrate	33 ± 2.9	4.7	14 ± 1.2
9	Growth hormone, 5 mg./Kg.	53 ± 6.4	4.7	10 ± 1.1
12	Methyltestosterone, 1 mg./Kg.	56 ± 2.5	5.1	40 ± 1.6
9	Androstenediol, 10 mg./Kg.	46 ± 3.1	4.4	24 ± 1.6
9	Methylandrostenediol, 1 mg./Kg.	47 ± 4.3	4.7	12 ± 1.2
9	Methylandrostenediol, 50 mg./Kg.	61 ± 3.5	5.1	66 ± 4.8

It will be noted that methylandrostenediol,[1] in a daily dose of 1 mg./Kg., or approximately 0.1 mg. per animal per day, produced an increase in weight of the levator ani muscle comparable to that produced by pituitary growth hormone or methyltestosterone. In this dose, no growth of the seminal vesicles occurred. It is of interest that androstenediol is slightly androgenic, as measured by its effect upon the seminal vesicles. It has already been noted by Deanesly and Parkes (5) that 17-methylation decreases the effect of this compound upon the seminal vesicles. Methylandrostenediol, in the inordinate dose of 50 mg./Kg., produced a moderate increase in the weight of the seminal vesicles. Histologic examination showed the growth to be true androgenic stimulation of the epithelium, and not the fibromuscular proliferation characteristic of an estrogenic

[1] 17(β)methyl-Δ5-androstene-3(β),17(α)-diol; formerly referred to as methyltrans-androstenediol.

ERRATA

In the article entitled "Methylandrostenediol: A Protein-Anabolic Steroid With Little Androgenic Activity" by G. S. Gordan, E. Eisenberg, H. D. Moon and W. Sakamoto, published in *J. Clin. Endocrinol.* 11: 209 (Feb.) 1951, the formula of the compound used should have been described as 17(α)-methyl-Δ^5-androstene-3(β),17(β)-diol.

effect. This observation is in harmony with those of Deanesly and Parkes (5), Ruzicka, Goldberg, and Rosenberg (6), Ruzicka and Rosenberg (7), Tschopp (8), Goldberg (9), and Dannenbaum (10) that methylandrostenediol is androgenic only in excessive dosage. This compound has also been assayed for other hormonal properties and found to be only weakly estrogenic (8) and deficient in progestational (11) and corticoid (12) activities.

Preliminary studies in 5 human female subjects have shown that methylandrostenediol in doses of 25 mg. per day produces an increase in weight with a concomitant retention of nitrogen and other protoplasmic constituents without masculinizing effects (13). Similar results have been reported in balance studies in 4 other human subjects by Thorn (14) and by Wilkins and Fleischmann (15). These investigators also commented on the lack of evidence of androgenic stimulation by methylandrostenediol.

SUMMARY

By use of the levator ani muscle of the castrate male rat as an index of protein-anabolic activity, methylandrostenediol was found to be a potent myotrophic agent in a dose of 1 mg./Kg. Androgenic properties were lacking at this dose and were found only with the inordinate dose of 50 mg./Kg. Preliminary studies have corroborated these effects in human female subjects.

Addendum

Since this manuscript was submitted for publication, our series of human subjects has been expanded to 46. Methylandrostenediol has been found to be effective when administered sublingually, and administration by this route allows better control than does intramuscular injection, since the latter results in the formation of depots. At this date (February 12, 1951) it is apparent that a dose of 40 mg. per day *sublingually* produces increase in weight, abolishes the pain and hypercalcuria of osteoporosis and of osteitis deformans, and does not produce virilization in adult women. A dose of 20 mg. per day *sublingually* administered to children has resulted in increment of height and weight without masculinization, but larger doses have occasionally produced mild androgenic effects. F. Homburger, S. H. Kasdon and W. H. Fishman (Methylandrostenediol: a non-virilizing derivative of testosterone in metastatic cancer of the breast, *Proc. Soc. Exper. Biol. & Med.* **74**: 162, 1950) have also reported effects of this compound upon mammary carcinomatosis, similar to those obtained with methyltestosterone but without virilization.

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OBSERVATIONS ON THE USE OF THE MALE NORTH AMERICAN FROG (*RANA PIPIENS*) IN PREGNANCY DIAGNOSIS

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THE male frog diagnostic test for early pregnancy, although widely used, is still a comparatively new procedure. Most reports to date are encouraging, citing the accuracy of this technic as approximately 95 per cent (1, 2, 3). As with any new procedure, however, certain difficulties have been encountered. Cutler (4), Brody (5) and Gardner and Harris (6) have encountered a high incidence of false negative reactions using the *Rana pipiens*. Most of their negative results were found on second and third trimester urines. In place of the original technic using 5 cc. of whole urine, they advocated the use of concentrates of larger volumes of urine. Samson has reported a considerable seasonal variation in the sensitivity of the *Rana pipiens* to chorionic gonadotropin. During the summer months he has found that the sensitivity falls to such levels, that for pregnancy diagnostic work the use of urine concentration methods is imperative (7). In addition, approximately 11 per cent of the frogs tested by this author proved to be "refractory" to pregnancy urine.

In our own laboratory, using 5 cc. of urine per frog in the routine test, death of the animals has blocked the performance of approximately 5 to 10 per cent of the tests. Approximately 20,000 male frogs have been used at the Mallory Institute of Pathology of the Boston City Hospital in the diagnosis of pregancy in the past three years. In this use of the amphibian certain difficulties have been encountered and it is the purpose of this report to present these problems and to suggest possible solutions. Some observations on the possible use of frogs for quantitative assay studies will also be presented.

Modifications of technic with particular reference to toxicity and sensitivity

One of the basic problems in any biologic pregnancy test is to select a volume of urine that avoids significant toxicity while achieving a high order of sensitivity. With the original technic of 5 cc. of whole urine per frog, a 96 per cent accuracy was achieved (3). However, a considerable mortality was encountered, which during the months of March and April reached a level of 15–20 per cent of the frogs used. These are the months

Received for publication July 14, 1950.

during which the frogs are in poorest physical condition, having been kept in enforced hibernation since the preceding summer. It is of interest that most of these toxic urines were found in patients with some abnormality of pregnancy, usually a threatened or an incomplete miscarriage. To avoid this mortality, two methods of detoxification of urine have been adopted: 1) concentration of the hormone by precipitation with alcohol; and 2) dialysis of the urine against running tap water. The first of these methods employs 20 cc. of urine per frog. The urine is shaken with 4 volumes of 95 per cent alcohol for one minute. The precipitate is collected by vacuum filtration and the paper with the deposited precipitate dried for two or three minutes. The precipitate is dissolved for injection by immersing the filter paper in 5 cc. of saline for ten minutes. With this method any volume of urine can be processed and considerable detoxification is accomplished. In a series of known toxic specimens, the redissolved precipitate was found to remain toxic in only about 1 out of 10 instances.

Serial dilution studies indicate that the per cent recovery of hormone with this technic varies in proportion to the original urinary level of hormone. High-titer urines yield recoveries of up to 80 per cent and thus the concentrate of 20 cc. of urine yields an amount of hormone equivalent to that present in 16 cc. of urine. With low-titer urines the percentage yield may not be more than 20 per cent of the hormone. It is at once apparent that urines of very early pregnancy having low titers give poor yields of hormone—less than the equivalent of 5 cc. of original urine. If for any reason, then, a urine is suspected of having a very low titer, it is desirable to use larger volumes of urine for the test dose. Fortunately, however, after the first few weeks of gestation the hormonal titer rises so rapidly that the amounts of hormone found in the usual diagnostic pregnancy urine are in such excess that this theoretic objection has little practical significance.

The results obtained with this technic have proven to be highly satisfactory. In 404 routine diagnostic tests performed, a 98 per cent accuracy has been achieved. The errors have been entirely false negative results. An analysis of these tests is presented in Table 1.

In an effort to eliminate toxic reactions more completely, dialysis of whole urine has been adopted. Reports by Heller and Chandler (8), and Heller and Heller (9) have indicated the usefulness of this method of detoxification in the concentration of urine for follicle-stimulating hormone (FSH) determinations. In their procedure, the urine precipitate is placed in a cellophane bag and dialyzed against running tap water. In the interests of keeping the frog technic as simple as possible, whole urine has been dialyzed rather than concentrates. A volume of urine, filtered if turbid, is placed into a cellophane bag produced by tying off the bottom of a length of cellophane tubing (DuPont cellophane dialyzing tubing)

and then tying off the top of the tubing just above the level of urine. This restriction of the urinary volume prevents dilution by absorption of water. After one hour's dialysis against running tap water the urine is ready for use. Five cc. is the commonly used test dose, although volumes up to 10 cc. have been used. Detoxification by this method is extremely efficient and more complete than with the previously described alcohol precipitation technic. Moreover, for weak-titer urines, dialysis appears to be preferable to precipitation. Control studies run on dialyzed and undialyzed urine reveal that no hormone is lost through the semipermeable membrane. In fact, as certain urines lose their toxicity they appear to gain in titer, as though the toxic substance may have indeed depressed the frogs' sensitivity. The number of tests studied to date with this technic is too small and

TABLE 1

Source of urine	Results of frog tests			
	Positive	Negative	False positives	False negatives
Normal pregnancy*	118	110	0	8
Probable or possible miscarriage	21	72	0	0
Menopause	0	12	0	0
Ectopic pregnancy	1	8	0	0
Others**	0	54	0	0

* These specimens were submitted for aid in the diagnosis of pregnancy, and thus represent, for the most part, first trimester urines.

** This group includes postoperative studies on moles, abdominal masses without pregnancy, simple amenorrhea, etc.

the follow-up too brief to have any more significance than strongly suggesting the possible value of this modification. The extremely high sensitivity of this technic may have, however, certain objections to be discussed.

Normal menstrual and menopausal urines

With urine samples derived from girls with regular menstrual cycles having had no period of amenorrhea or from women entering the menopause, an occasional false positive reaction has been encountered. These positive reactions have been encountered throughout the menstrual cycle in random fashion. Most of them have occurred using the dialyzing technic described. All attempts at elucidating these reactions have failed, except that the substance causing them does not appear to be of ovarian origin

or to be gonadotropin. The fact that the male frog will react to pituitary FSH may explain why a rare menopausal urine evokes gametokinesis.

The few postmenopausal urines studied have not given similar positive reactions.

Although these non-pregnancy positive reactions are mentioned here as theoretic dangers, in the actual practice of our pregnancy diagnostic work only 3 false positive reactions have been encountered in over 3,000 diagnostic tests, 1 of which has already been reported. However, out of regard for this potentiality, it is our practice to obtain a brief history on all urine samples submitted and to refrain from performing frog tests on specimens from patients not having amenorrhea or on menopausal women. When necessary, such tests are performed on rats or rabbits. Happily, the substance or substances responsible for producing these positive reactions are found only in amounts barely sufficient to evoke a positive response; neither do they produce reactions in all of the animals used. The greatest danger of encountering such false positive reactions appears to be present when the dialysis technic is used during the frogs' breeding season of March April and early May when they are most susceptible to stimulation.

Seasonal variations in frogs

Several reports have appeared in the recent literature referring to the seasonal variation in the sensitivity of the male frog to gonadotropin. Samson cites the sensitivity as varying from the 2 to 5 I.U. of chorionic gonadotropin required to produce a reaction normally, to the 10 to 25 I.U. required during the insensitive summer season (7). Moreover, as has been cited, 11 per cent of the amphibia used by him were totally "refractory" to gonadotropin. Although seasonal variation in sensitivity to adrenalin has been observed, the experience at this laboratory has not indicated any change with relation to the gonadotropins (10). Any evaluation of sensitivity changes in frogs must be based on the premise that frogs vary in sensitivity from one animal to the other and that climactic conditions and conditions of holding them seriously affect this sensitivity. Such individual differences may account for the failure of all frogs to react similarly to a critically low urinary titer of gonadotropin. However, in our experience, the constancy throughout the year of the sensitivity of large numbers of frogs to any given level of gonadotropin has been quite remarkable when tested by a constant dose of a stable, dried preparation of gonadotropin. Further corroboration of this seasonal stability is derived from an analysis of routine pregnancy diagnostic tests. Performed under identical conditions, they fail to disclose any increased incidence of false negative results during any particular season. On the contrary, as was mentioned earlier, increased sensitivity has at times been a problem. During the mating

season of March, April and May occasional animals taken directly from the storage tanks will show sperm in their cloacal fluid after simple handling. During this season the spermatic ejaculatory response appears to be highly unstable and somewhat nonspecific. It is therefore necessary during these months to examine all frogs prior to use. Storage of these frogs in cold water for approximately four to five days after their arrival in the laboratory seems to diminish greatly this nonspecific reactivity. It is during this period of heightened reactivity that normal menstrual and menopausal urines are particularly prone to give positive reactions. These observations indicate the need for caution in the use of these amphibia as test animals, especially in the breeding season. However, the rarity of false positive reactions reported by most laboratories with the use of the *Rana pipiens* attests to the insignificance of these complications as practical problems (6).

Quantitative studies

Reference has already been made to the remarkably constant sensitivity of these male frogs to gonadotropin. Repeated experiments have shown that most frogs react to a virtually constant level of hormone (11). The end point for macroscopic corpora lutea in the immature white rat is, within narrow limits, produced by the same volume of urine that will just produce a positive reaction in the frog, hence the minimum reactive dose is practically equal for frog and rat.

These observations strongly suggested the possible use of the male frog for quantitative gonadotropic assay. A series of such assays performed on urines from both normal pregnancy and complications of pregnancy are listed in Table 2, indicating the comparative titers as determined on frogs and rats.

DISCUSSION

All reports agree that false positive results are extremely rare in the male frog pregnancy test. However, there is considerable difference of opinion as to the sensitivity of this test, varying from a low of 44 per cent false negatives (Gardner and Harris) to results citing an error of only 2 to 4 per cent false negatives. A subsequent series of 52 urines analyzed by Gardner and Grady indicates considerable improvement in results, stemming from better conditions of maintaining the frogs. The false negative results in this last series were attributed to abnormalities of pregnancy and to very low titers of hormone present in the first six weeks of gestation. It is also likely that many of the poor results reported may stem from the inclusion within the test series of large numbers of second and third trimester pregnancy urines having low gonadotropic titers. Such low levels of hormone may give false negative results with any diagnostic procedure.

In our hands, toxicity, not insensitivity, has been the chief problem. Methods of concentration of the urine and/or dialysis of whole urine have successfully overcome this problem. The concentration technic has proven eminently satisfactory as far as accuracy is concerned (2 per cent false negative errors in 404 tests). It, however, is subject to the problem of decreasing yields as the titer of the original urine falls. The 8 false negative

TABLE 2. QUANTITATIVE TITERS

Specimen source (urines, 24-hour specimens)	Frogs, units/liter	Rats, macroscopic corpus luteum units/liter
3 Months' pregnancy	1,000	1,000
Uterine chorionepithelioma	50,000	50,000
Uterine chorionepithelioma	20,000	20,000 (positive) 40,000 (weakly positive)
3½ Months' pregnancy	2,000	1,000
3½ Months' pregnancy	400	200 (positive) 400 (weakly positive)
4 Months' pregnancy	200	200
Early pregnancy (spot specimen)	1,000	500
Early pregnancy	500	500–1,000 (weakly positive)
Early pregnancy (spot specimen)	10,000	10,000
Early pregnancy	1,000	1,000
2 Months' pregnancy	60,000	60,000 (negative*)
2½–3 Months' pregnancy	30,000	30,000 (negative*)
4 Months' pregnancy	400	200
4 Months' pregnancy	400	1,000
2 Months' pregnancy	2,000	400
5 Months' pregnancy	140	200
3½ Months' pregnancy	1,200	600
? Duration of pregnancy	2,000	1,200 (positive) 4,000 (weakly positive)
3 Months' pregnancy	500	400
4 Months' pregnancy	500	400 (negative)*

* Titers below this level were not determined.

tests were encountered in 6 early pregnancies in some of which menstruation was overdue less than fourteen days) and in 2 late pregnancies in the second or third trimesters of gestation.

Dialysis appears to bear great promise as a detoxification method, since no hormone is lost in this manipulation and the frogs tolerate up to 10 cc. of urine per animal with very rare exceptions. The word of caution spoken with relation to the menopausal urines and urines from normally cyclic girls emphasizes the need for correlating all pregnancy diagnostic work with a clinical history. It would be entirely possible in the absence of clini-

cal data to render a false positive report on such urines, especially during the breeding season of these amphibia. It is to be emphasized, however, that the likelihood of this possibility is quite remote, since these urines contain the reactive substance in such very small amounts that, at most, one isolated positive reaction might be encountered in one of several frogs.

The use of the frog test for quantitative hormonal assay has many distinct advantages. The male frog unit appears to be essentially identical to the rat unit as determined by the production of macroscopic corpora lutea. If microscopic sections of the rat ovaries are prepared and microscopic evidence of corpus luteum formation taken as the end point, then the rat will be found to react to smaller amounts of gonadotropin than the frog. In any event, the difference is so small as to have little clinical significance. At worst, having a fairly close estimation of the titer of the urine in the frog, it is a simple matter to corroborate this end point with rats.

With frogs, a quantitative titration can be completed in three to four hours and the result transposed, if desired, within reasonably close limits into rat units.

SUMMARY AND CONCLUSIONS

Two modifications in the technic of the performance of the male frog test for pregnancy are described. The one concentrates 20 cc. of urine per frog by precipitation with 4 volumes of 95 per cent alcohol. The other uses dialysis of 5 cc. of urine per frog as a means of lowering the toxicity of whole urine. The advantages of each method are discussed. A series of 404 tests performed with the alcohol concentration method yielded an over-all accuracy of 98 per cent with no false positives.

Some of the problems inherent in the use of these frogs during their breeding season are presented. The increased reactivity at this time coupled with some unknown substance found in normally cyclic and menopausal women together produce a theoretic hazard of false positive reactions. Possible means of avoiding this complication are presented and with these precautions the real danger of false positive results is apparently very slight, since in approximately 3,000 routine diagnostic tests only 3 have ever been encountered. In these amphibia, no seasonal variation of sensitivity to gonadotropins has been encountered at this laboratory. On the contrary, a remarkably constant level of sensitivity has been found to be the case, so that quantitative studies are readily performed with the male frog. Work to date indicates that the male frog and the immature 21-day white rat respond to virtually identical amounts of chorionic gonadotropin.

Acknowledgment

We are indebted to Ciba Pharmaceutical Products, Incorporated, Summit, New Jersey, for the support of this work.

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PITUITARY INFANTILISM TREATED WITH PURIFIED GROWTH HORMONE, THYROID, AND SUBLINGUAL METHYLTESTOSTERONE. A CASE REPORT*†

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THE availability of growth hormone of the anterior pituitary in electrophoretically homogeneous and biologically pure form seemed to promise adequate substitution therapy for hypophyseal infantilism (pituitary dwarfism). Furthermore, the hormone was of high potency in the laboratory; the minimal effective dose in hypophysectomized rats was only 10 gamma (1). This fact raised hope that larger amounts might be effective in the human disorder that was the counterpart of the picture seen in hypophysectomized rats. Previous unsatisfactory experience with a less pure growth hormone preparation could have been due to a relatively low potency.

The patient reported here seemed a typical example of true hypophyseal infantilism and was thought to offer an excellent opportunity for testing growth hormone[1] in adequate amounts in a human being. The experiment was both clinical and chemical, the former for actual amount of linear growth and effect on bone maturation, and the latter for changes in the nitrogen, calcium and phosphorus balances.

As will be shown in the following report, the results of therapy with growth hormone proved disappointing. Other substances known to affect linear growth (thyroid and methyltestosterone) were then tried for comparison, and later a combination was administered. Estrogens were not used because of their known tendency to close epiphyses.

CASE REPORT

F. H. (University of California Clinic No. U 132309) was first seen on September 4, 1946, at the age of 16 years and 8 months. Her chief complaint was shortness of stature.

Received for publication July 10, 1950.

* Read at the Annual Meeting of the Association for the Study of Internal Secretions, San Francisco, California, June 23, 1950.

† Help in this study was received from the Ciba Fund for Endocrine Investigation.

[1] The growth hormone used was prepared and made available by Dr. C. H. Li, according to the method of Li, Evans, and Simpson (1). The hormone was sterilized by centrifugalization in a Hopkin's tube, keeping the pH at 10 to 11, and finally by pressure filtration through a collodion membrane. We are grateful for the help of Dr. Alfred S. Lazarus and Dr. Gordon Meiklejohn in these matters.

The history revealed that she was the only child of parents who were relatively tall. Her father was 6 feet in height, of Austrian origin, and her mother was 5 feet, 8 inches in height, of American birth and extraction. One paternal cousin had been short, reaching a height of only 4 feet, 8 inches in maturity.

The patient, who was born in China, seemed to grow and develop normally until the age of 5 years, at which time a definite slowing of growth was noted. When she was 6 years old, medical advice was sought. Her height was then $40\frac{1}{2}$ inches (103 cm.). She was started on a thyroid preparation given by injection, which was administered off and on for a period of two years. During this time the patient grew about 2 inches a year, and at the age of 8 years was $44\frac{1}{2}$ inches (113.5 cm.) in height. Therapy was then changed to injections of pituitary extract and an oral pituitary preparation (Eliteran). Growth continued, but at a gradually decreasing rate; three years later at the age of 11 years, her height was $48\frac{1}{2}$ inches (123 cm.). At this time she was examined at the Peiping Union Medical College Hospital, where it was found that her basal metabolic rate was minus 30 per cent and the plasma cholesterol 330 mg. per cent. Her bone age was 10 years (at $11\frac{1}{2}$) and studies of the sella turcica showed it to be questionably, slightly enlarged. Her intelligence quotient was 129. Treatment was changed to injections of Anterior Pituitary Extract (Squibb), 1 cc. three times a week; and thyroid substance, .065 to 0.09 gram daily. This dosage did not appreciably affect the rate of growth and was discontinued after a few months because of war conditions.

At the age of 15 years, the patient's height was $50\frac{3}{4}$ inches (129.5 cm.). The rate of growth had gradually slowed, and apparently had stopped one year previously. She was then seen by Professor B. Pribam, who advised the trial of a pituitary transplant. Accordingly, in June, 1945, two pituitary glands of freshly slaughtered calves were grafted between the parametria. While the abdomen was open, he noted that the uterus seemed completely absent and that small ovaries, each the size of half a peanut, were present (2). The patient was also given thyroid substance for a time, but no growth resulted, as our first measurement a year and a half later was less than the height reported before the operation. She had always been mentally above average, graduating from high school at $16\frac{1}{2}$ years and placing second in her class.

When first seen at the University of California Clinic at the age of 16 years and 8 months, her height was $49\frac{1}{2}$ inches (126 cm.) and her weight $67\frac{1}{2}$ pounds (30.7 kilograms). She was well proportioned and slightly plump (Fig. 1A). There was no breast development and the external genitalia were prepuberal. No uterus or adnexae could be felt by rectal examination. The arm and leg lengths were proportional to the height, the pulse rate was 68 a minute and the blood pressure was 120/80.

Special studies showed the following: The sella turcica was somewhat wide but was not suggestive of a tumor. The visual fields were normal. The bone age was 13 years and the dental age 14 years. A glucose tolerance test (Somogyi method) gave these results: fasting, 73 mg. per cent; 1 hour, 107 mg. per cent; 2 hours, 76 mg. per cent; 3 hours, 85 mg. per cent; and 4 hours, 71 mg. per cent. The basal metabolic rate was minus 22 per cent; plasma cholesterol, 254 mg. per cent, and the serum carotin showed no excess. Follicle-stimulating hormone (FSH) excretion in the urine was below 6.4 mouse units in twenty-four hours, and 17-ketosteroid excretion (ketonic fraction) was 1 mg. in twenty-four hours.

It was decided to try the effect of purified growth hormone on this patient. Accordingly, she was admitted to the University of California Hospital on December 1, 1946, for balance studies. A constant liquid diet was administered and daily diet and urine analyses were made for nitrogen, calcium and phosphorus. Stools were pooled for control

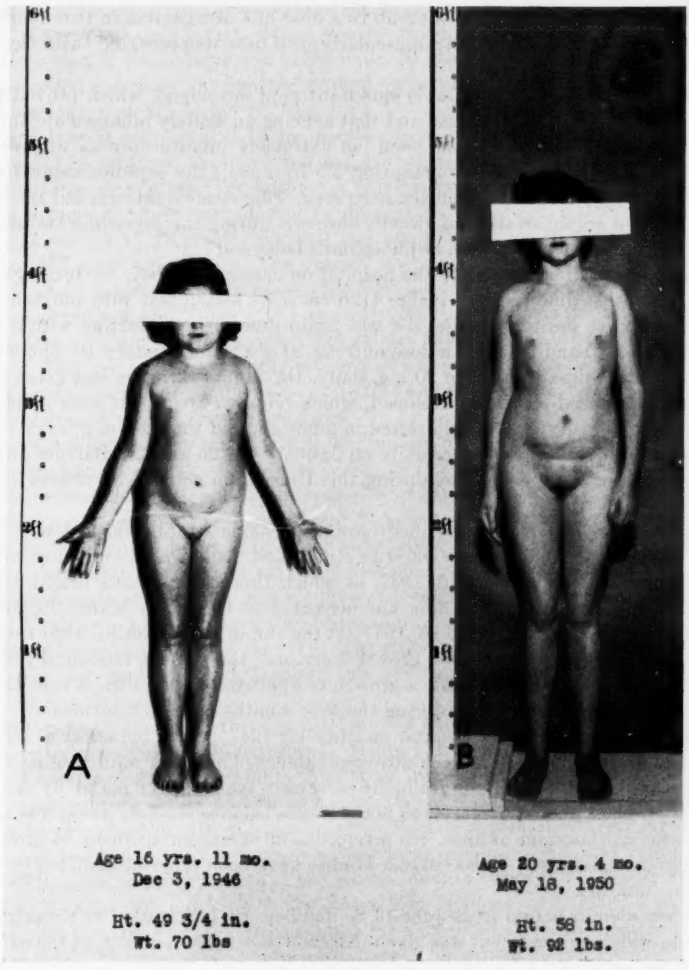

FIG. 1.

A. Appearance of patient at start of treatment when there had been no growth for a period of two and one-half years.
B. Appearance after three and one-half years of treatment and total growth of $8\frac{1}{4}$ inches, most of which had resulted from combined therapy with thyroid substance and methyltestosterone linguets.

and treatment periods and were analyzed for the same constituents. Blood levels were also estimated for calcium, phosphorus, phosphatase, nonprotein nitrogen and serum proteins. Since the growth hormone is a protein, the patient was skin-tested before administration and was found to be sensitive. She was desensitized, starting with a dose of

1 gamma subcutaneously and working up to a dose of 1,400 gamma in two hours. Later that day she was given 3.5 mg. intramuscularly; this dose was repeated twice the second day.

On the third day some right lower quadrant pain developed, which proved to be a typical attack of acute appendicitis, and that evening an acutely inflamed appendix was removed. While the abdomen was open, an extremely infantile uterus was observed. Both ovaries were present, each measuring 2.5 by 1 cm.; the capsules seemed slightly thickened and no well-formed follicles were seen. This episode interrupted the balance studies, but the administration of growth hormone during the preceding two days had not affected the nitrogen, calcium or phosphorus balances.

The patient was readmitted to the hospital on January 6, 1947, for further studies. Her height at this time was $49\frac{3}{4}$ inches (126 cm.). As a skin test with purified growth hormone still gave positive results, she was again desensitized, starting with a dose of 1 gamma at 8 A.M. and reaching a dose of 5 mg. at 8 P.M. on January 16. She was then able to tolerate a dosage of 10 to 20 mg. daily. On January 22, she was given 40 mg., but a giant urticarial reaction developed, which required treatment with epinephrine. The next day Benadryl was administered in addition, and the dose of growth hormone was gradually increased to 60 mg. daily on January 25, 26 and 27. Balance studies of nitrogen, calcium and phosphorus during this time again showed no change from the treatment.

It was then decided to give purified growth hormone steadily for a period of three months to see if the linear growth could be stimulated. An intramuscular dose of 10 mg. daily was started on February 10, 1947, at which time the patient's height measured $50\frac{1}{8}$ inches (128 cm.). The daily dose was increased to 15 mg. on March 20, to 20 mg. on April 5, and to 25 mg. on April 26, 1947. At the end of three months, after receiving a total dose of 1,360 mg. of purified growth hormone, the patient measured $50\frac{1}{2}$ inches (129 cm.) in height. This represents a growth of approximately $\frac{3}{8}$ inch, a rate of growth not appreciably in excess of that during the four months prior to treatment.

She was readmitted to the hospital on May 12, 1947, to try the effect of still larger amounts of growth hormone on the nitrogen balance. Following reduction to 10 mg. a day for a few days, the dose was gradually increased, reaching 160 mg. daily on May 30 and May 31. Once again no effect was noted in the balance studies. Blood was taken at this time for antihormone studies. No precipitins or effects antagonistic to growth hormone were demonstrated. These balance studies have been reported in detail in another communication (3).

The three-month period from June to September, 1947, was used as a control interval, during which no treatment was given. No growth occurred; in fact, at the end of this time the patient's height measured $50\frac{3}{8}$ inches (128.5 cm.), slightly less than at the end of the period of growth-hormone therapy.

Next, a three-month period of therapy with desiccated thyroid was tried. The dose was prescribed initially at .03 gram daily, later at .065 gram daily, but still later had to be reduced to .045 gram daily because of nervousness and palpitation. At the end of three months of treatment with thyroid, the patient's height was $50\frac{3}{4}$ inches (129.5 cm.), another gain of approximately $\frac{3}{8}$ inch. A repeated study of epiphyses at this time showed no evidence of maturation, the bone age still being 13 years.

Treatment was then withheld for one month, until January 15, 1948, when, shortly after her eighteenth birthday, the patient was started on methyltestosterone, given as 5-mg. methyltestosterone linguets[2] once daily. At this time her height was $50\frac{7}{8}$ inches

[2] The methyltestosterone linguets used were Metandren linguets kindly furnished by the Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

(129.7 cm.). After three months of therapy the height was 51¼ inches (131 cm.), another increase of approximately ⅜ inch. There were no masculinizing effects from this dosage. Then, for a month the patient was given daily 20 mg. of methyltestosterone linguets. This dosage did not cause any spurt of growth, in fact during that month the height remained stationary at 51¼ inches (131 cm.).

At that time it was decided to try combination therapy to see if a faster rate of growth could be achieved. No more purified growth hormone was available, so thyroid and testosterone were given. The latter was administered as methyltestosterone, 10 mg. daily, in a linguet which was allowed to dissolve in the buccal gutter. Thyroid substance was started at .03 gram daily and was gradually increased over a period of six weeks to .065 gram twice daily. The patient was apparently able to tolerate a larger dose of thyroid substance while taking testosterone than had been possible when she was given thyroid alone. There was a considerable spurt in growth and at the end of three months of combined therapy her height was 52⅝ inches (134 cm.), an increase of 1⅜ inches. Therapy for three months on each of the previous regimens had resulted in growth of only ⅜ inch. The combination of thyroid and methyltestosterone was continued and the increased rate of growth persisted.

After nine months (February 3, 1949), the patient's height was 4 feet, 6⅝ inches (139 cm.), representing a growth of 3⅜ inches in this period. During this time various studies had been made. Follicle-stimulating hormone (FSH) excretion tests gave negative results at 3 mouse units and 2.5 mouse units, and 17-ketosteroid excretion was 1.6 mg. (total neutral) in twenty-four hours. The basal metabolic rate was plus 14 per cent, plasma cholesterol was 277 mg. per cent and protein-bound iodine in the blood was 8.6 micra. The bones were larger, but no new epiphyseal fusions had occurred and the bone age was still 13 years. There had been slight masculinization, with some hair growth on the upper lip and some huskiness of the voice. The clitoris seemed somewhat prominent and a few pubic hairs had appeared. None of this was marked enough to cause the patient any discomfort or concern.

A decision was made to try therapy with thyroid substance alone for two months, and the methyltestosterone was discontinued. Within two weeks the patient complained of increased nervousness and irritability, and some increase in pulse rate was noted. The dosage of thyroid substance had to be cut to .03 gram, three times daily. During this time her basal metabolic rate was plus 2 per cent; plasma cholesterol, 247 mg. per cent; and protein-bound iodine, 6.1 micra. Another measurement of follicle-stimulating hormone (FSH) gave negative results at 2.5 mouse units in twenty-four hours. At the end of the two months, the patient's height was 4 feet, 7¼ inches (141 cm.), a further gain of ⅝ inch. All therapy was then discontinued for a two-month control period. Again the growth stopped, the height remaining stationary. The patient became drowsy, felt cold and noted some dryness of the skin and slowing of mental reactions. At the end of the control period, the basal metabolic rate was minus 23 per cent; plasma cholesterol, 111 mg. per cent; and protein-bound iodine, 2.6 micra. Follicle-stimulating hormone (FSH) excretion was positive at 3 mouse units and 17-ketosteroid excretion was 0.7 mg. (total neutral) in twenty-four hours.

On June 4, 1949, treatment was resumed with the same medication (methyltestosterone linguets, 10 mg. once daily and thyroid substance, .065 gram twice daily). Growth resumed, and six months later the patient's height had increased to 4 feet, 8⅝ inches (144 cm.), a further increase of 1⅜ inches. Since the rate of growth was less than that achieved previously, the dosage of thyroid substance was increased to .065 gram, three times daily. This dosage was continued for another five months until May 18, 1950, at which time the patient's height had reached 4 feet, 10 inches (147 cm.) (Fig.

1B). This represented a further growth of $1\frac{3}{8}$ inches, and a total growth of $8\frac{1}{2}$ inches in the three years and eight months of observation and treatment (Fig. 2). Another estimation of bone age at this time showed a level of 13 years. There was still no maturation of the epiphyses from the treatment to date, although the bones were larger.

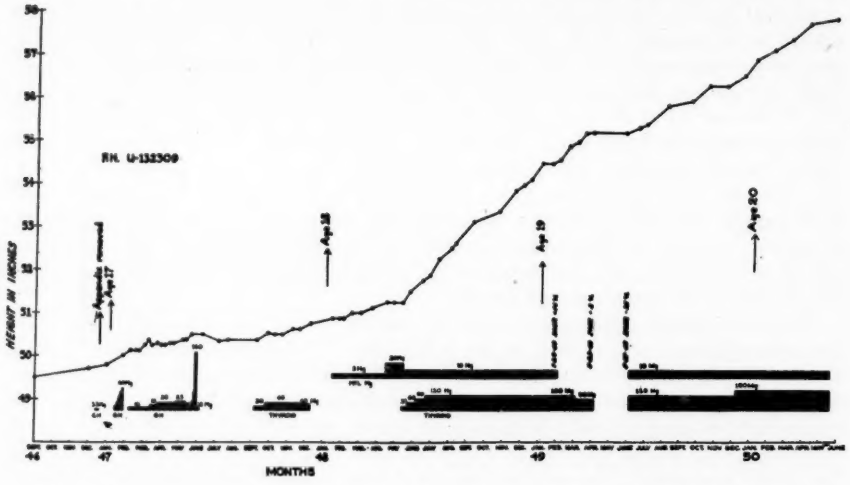

FIG. 2. Growth curve during period of observation. Treatment with purified growth hormone (GH), thyroid substance and methyltestosterone linguets (MTL) is indicated.

DISCUSSION

From the preceding data it appears that purified growth hormone may have caused slight stimulation of linear growth in this patient, but with dosages of 160 mg. daily there was no apparent effect on the nitrogen, calcium or phosphorus metabolism. Many questions are suggested by this result. Was the dose adequate? Is the bovine growth hormone the same growth hormone that the human pituitary elaborates? Did the lack of favorable response represent a failure of end-organ receptivity? Since the patient did grow on combined therapy with thyroid substance and methyltestosterone, should not the combination of thyroid substance and growth hormone have been tried? Our data do not answer the above questions.

The patient was not considered to have primary hypothyroidism, despite the low basal metabolic rate and slightly high cholesterol values. Against this also was her alert appearance and high intelligence quotient and the failure to grow dramatically on thyroid alone. Furthermore, the thyroid therapy given to date has not caused any acceleration in the bone age.

Methyltestosterone was used because of its known effect in stimulating linear growth in boys (4, 5), and because it does not hasten epiphyseal

closure as do estrogens. No skeletal maturation has occurred in this patient after three years and eight months of therapy, during two years and four months of which, testosterone was administered. It is planned to employ estrogens later, after maximum growth has been achieved and sexual advancement becomes desirable. The dosage of testosterone used did cause some evidence of masculinization, with huskiness of the voice, slight hair growth on the upper lip, and slight prominence of the clitoris. The symptoms were never annoying enough to bother the patient materially, especially in view of the growth achieved. Methyltestosterone linguets were chosen, since this method of administering testosterone is simple, efficient and economical.

The apparent synergistic effect of testosterone and thyroid substance when administered simultaneously is not readily explained, although similar effects are noted in hypophysectomized rats (unpublished data).

SUMMARY

A female patient with hypophyseal infantilism is described. She was 16 years and 8 months old when first seen and her height was 49½ inches. Previous treatment with thyroid substance, pituitary tablets and injections, anterior pituitary extract and thyroid substance, and an implant of fresh calves' pituitary glands had not produced any significant growth.

Purified growth hormone was administered in doses up to 160 mg. a day without affecting the nitrogen, calcium or phosphorus balance of the patient. Treatment for three months with growth hormone in doses of 10 to 25 mg. daily, administered intramuscularly, was associated with a growth of only approximately ⅜ inch. After a control period, the administration of thyroid substance alone was associated with a growth of approximately ⅜ inch in three months. After another control period, the administration of methyltestosterone linguets in a dose of 5 mg. daily for a similar period of three months, likewise was associated with a growth of ⅜ inch. However, on a combination of methyltestosterone linguets (10 mg. daily) plus thyroid substance (.065 gram twice daily) there was a definite spurt in growth, manifested by an increase of 1⅜ inches in three months. Continued therapy with this combination, with occasional interruptions for control periods, has been given so far for approximately two years; and the patient has reached a height of 4 feet, 10 inches, an increase of 6¾ inches during this time. Total growth of 8½ inches has now been achieved without causing any advance in "bone-age" maturity.

Questions are raised concerning the reasons for the disappointingly slight effect of purified growth hormone and the apparent synergistic effect of the thyroid substance and methyltestosterone administered simultaneously.

Acknowledgment

The assistance of Dr. Howard J. Weinberger and Dr. Sheldon Margen is gratefully acknowledged. Their contribution of careful supervision of the patient during her periods of hospitalization and the early part of her treatment was invaluable.

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The Association for the Study of Internal Secretions

LETTER TO THE EDITOR

To the Editor:

As the Chairman of the Program Committee for the meeting in Atlantic City, Thursday, Friday and Saturday, June 7, 8, and 9, I am writing to give a few more details regarding our plans.

At present we are planning that on Thursday morning at 9:30 there be a program on "General Subjects" with Drs. C. N. H. Long and Lawson Wilkins presiding; at 2:00 p.m. "Papers Relating to the Pituitary" with Drs. Jane Russell and J. S. L. Browne presiding; and in the evening, "Information Please" with Dr. E. H. Rynearson presiding.

For the "Information Please" program, those attending the meeting are encouraged to send in any questions which they believe will prove of interest to other members. It is hoped that some of these questions will arouse discussion and perhaps controversy. At the present time it is not planned to have any members designated to take part in a panel. Doctor Rynearson plans simply to read the question and then call upon individual members for extemporaneous discussion. It is hoped that this program will be both informative and informal.

On Friday morning we plan to start at 9:00 with "Papers Relating to the Thyroid and Parathyroids" with Drs. Rulon Rawson and Robert Williams presiding. On Friday afternoon the program will consist of "Papers Relating to Gonads and Reproduction" with Drs. Earl Engle and E. Hamblen presiding. Friday evening there is the banquet. Saturday morning there will be "Papers Relating to the Adrenals" with Drs. Gregory Pincus and George Thorn in charge. On Saturday afternoon at 2:00 is a joint session with the American Diabetes Association, presided over by Drs. E. H. Rynearson and Lester Palmer. At 4:30 p.m. the Annual Business Meeting will be held.

JANE A. RUSSELL, Ph.D.

THE 1951 ANNUAL MEETING

The Thirty-third Annual Meeting of the Association for the Study of Internal Secretions will be held at the Chalfonte-Haddon Hall Hotel, Atlantic City, New Jersey. The Scientific Sessions have been extended to three days, Thursday, Friday, and Saturday, June 7-8-9.

The Local Chairman of Arrangements is Dr. Matthew Molitch.

Due to the advance in the date of our meeting, Chalfonte-Haddon Hall Hotel will be unable to accommodate all of our members by reason of a previous commitment. The Colton Manor, on Pennsylvania Avenue near Haddon Hall, is an excellent hotel, which has been recently refurnished, and is holding one hundred rooms for our members on the "first come, first served" basis. The Hotel Strand, at Pennsylvania Avenue and the Boardwalk, has also promised to supply a limited number of rooms. It is important that reservations be made immediately if you are to avoid disappointment.

All Scientific Sessions will be held in the Chalfonte-Haddon Hall Hotel. The rooms, in which the session will be held, will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, June 8, at 7:30 P.M. in the Rutland Room, preceded by cocktails at 6:30 P.M. in the adjoining Benjamin West Room.

Those wishing to present papers, which will be limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Jane Russell, School of Medicine, Emory University, Atlanta, Georgia, not later than *March 1, 1951. It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and journals of the Association.* The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each non-member author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of non-members who are introduced, *i.e.*, who are not collaborators with member-authors,

are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.

Line 3. Institution of origin and city in which institution is located.

3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter-perfect, since there will be no opportunity for proof reading by authors.

(For details about the type of program, *see* the President's Letter to the Editor on page 1355 of the October 1950 issue.)

THE 1951 AWARDS AND FELLOWSHIPS

Fellowships

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship will not be awarded in 1951, so that it may accumulate toward a $5,000.00 Fellowship to be awarded in 1952.

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

The Schering Fellowship in Endocrinology was established in 1949 and the first recipient was Dr. D. Laurence Wilson, who also received the 1950 Fellowship.

Association Fellowships are designed to assist men or women of exceptional promise in their progress toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $2,500.00, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with

whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

Awards

THE E. R. SQUIBB AND SONS AWARD

The E. R. Squibb & Sons Award of $1,000.00 was established in 1929, and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr E. A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; in 1949 to Dr. Herbert M. Evans; and in 1950 to Dr. C. N. H. Long. In 1943 no award was given. A special committee of five members of the Association selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

THE CIBA AWARD

The Ciba Award to recognize the meritorious accomplishment of an investigator not more than thirty-five years of age in the field of clinical or preclinical endocrinology was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; in 1949 to Dr. George Sayers; and in 1950 to Dr. Oscar M. Hechter. The Award is for $1,200.00. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $1,800.00.

NOMINATIONS

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in, endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on special application forms which may be obtained from the *Secretary, Dr. Henry H. Turner,* 1200 North Walker Street, Oklahoma City 3, Oklahoma, and returned to him *not later than March 1, 1951.*

FOURTH ANNUAL POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY INCLUDING DIABETES

Sponsored by

THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

and

THE AMERICAN DIABETES ASSOCIATION

SEATTLE, WASHINGTON OLYMPIC HOTEL JULY 2–7, 1951

The faculty will consist of prominent researchers and clinicians in the field of endocrinology and metabolic disorders.

The course will be a practical one of interest and value to the specialist and those in general practice. The program will consist of lectures, clinics, and demonstrations. Ample time will be given to questions and answers at the end of each session, and registrants are encouraged to contact members of the faculty for individual discussions.

The Olympic, one of Seattle's most delightful hotels, offers special convention rates to members of this assembly. This is an unusual opportunity for you and your family to enjoy a pleasant vacation in the beautiful Pacific Northwest and for you to participate in a highly instructive program of the latest advances in endocrinology and metabolism.

A fee of $75 will be charged for the entire course and the attendance will be limited to 100. REGISTRATION WILL BE IN THE ORDER OF CHECKS RECEIVED AND WILL CLOSE ON JUNE 4, 1951. SHOULD THERE BE AN INSUFFICIENT NUMBER OF APPLICANTS TO FILL THE COURSE, THE REGISTRATION FEE WILL BE REFUNDED IMMEDIATELY IN ITS FULL AMOUNT.

Please forward application on your letterhead, together with check payable to The Association for the Study of Internal Secretions, to Henry H. Turner, M.D., Secretary-Treasurer, 1200 North Walker Street, Oklahoma City 3, Oklahoma, *before June 4, 1951*. Further information and program will be furnished upon request.

Hotel reservations should be made directly with the Olympic Hotel, Seattle, Washington, and the hotel advised that you are attending this Postgraduate Assembly.

The American Goiter Association

THE 1951 ANNUAL MEETING

THE 1951 annual meeting of the American Goiter Association will be held in Columbus, Ohio, on May 24, 25, and 26. The Deshler-Wallick Hotel, Broad and High Streets, will be the headquarters and every effort will be made by the hotel officials to make the meeting a success and the visit a very pleasant one. It would be well to see that reservations are made now.

The president, Dr. T. C. Davison, and the chairman of the Local Arrangements Committee, Dr. George Curtis, are planning an excellent meeting which will consist of papers dealing with goiter and other diseases of the thyroid gland, dry clinics and demonstrations. It is hoped that there will be a large attendance of members.

GEORGE C. SHIVERS, M.D.,
Corresponding Secretary,
Colorado Springs, Colo.

VAN METER PRIZE AWARD FOR 1951

THE American Goiter Association again offers the Van Meter Prize Award of three hundred dollars, and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association which will be held in Columbus, Ohio, May 24, 25, 26, 1951, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy in duplicate sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East Saint Vrain Street, Colorado Springs, Colorado, *not later than March 1, 1951.* The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award Essay by the author, if it is possible for him to attend. The essay will be published in the annual Transactions of the Association.